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THE TOXICOLOGY OF BERYLLIUM

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I. INTRODUCTION

The increased production and use of beryllium in industry has engaged the attention of a few hygienists who have been concerned about exposure of workmen to dust and fumes arising from the processing of this metal (*38*, *46*, *130*). Within the past decade several investigators have pointed out that the reduction of beryllium ore is hazardous from the point of view of poisoning, and others have found beryllium to be the cause of a rachitic condition. A certain amount of literature has been accumulated with reference to "beryllium poisoning," "berylliosis," and "beryllium rickets." The purpose of the present investigation was to measure and define the toxicity of beryllium and to indicate, so far as is feasible by means of animal experiments, the maximum permissible concentration of dust or fumes from the metal or its compounds which workmen should be permitted to breathe.

The economic importance of beryllium is out of all proportion to the tonnage produced. This arises not so much from the difficulty of producing the metal, as from the circumstance that the exceedingly valuable properties of the metal in the form of certain of its alloys have only recently engaged the attention of the metal industry (*4*, *70*, *106*). Vauquelin (*123*) discovered beryllium in 1797 and named it glucinium owing to the curious sweet taste associated with its salts, but the metal was not isolated until many years later and until comparatively recently it was merely a laboratory curiosity. While the metal has received two names, glucinium and beryllium, the former of which is in current use in France and is to be preferred on historical grounds, the name beryllium is more commonly used in this country. Beginning with the systematic investigation of the structure of beryllium-copper alloys made by Oesterheld (*93*) in 1916, the study of the effect of beryllium on copper by Corson (*21*) in 1926 and further work by Masing and Dahl (*79*), in 1928, the importance of beryllium in industry has greatly increased.

Although beryllium is found in some 14 different minerals in amounts varying from 10 to 53 percent beryllium oxide (*112*), the principal beryllium ore is beryl (14 percent BeO). Beryl is widely distributed (*113*) but occurs only rarely in deposits sufficiently large to be commercially workable. According to Tyler (*121*) domestic

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production of beryl prior to 1940 has probably not greatly exceeded 100 tons in any one year. While the present production figures are not available, the imports of beryl rose to 1,635 short tons during the first 9 months of 1941 (*80*). Tyler states that the imports for 1940 were 805 short tons; for 1939, 459 tons; while for 1938 the total amount imported was only 146 short tons. It has been estimated that the output of beryllium compounds and alloys in 1940 exceeded $500,000 in value. While these output figures seem small in comparison with the output of many other metals, it should be noted that 1 ton of beryl containing only 5 per cent of beryllium will produce enough beryllium to make 3,000 to 8,000 pounds of alloy suitable for the manufacture of parts of precision instruments (*122*). The price of beryllium has progressively dropped from $200 per pound in 1929 to $15 per pound of master alloy content in 1941 (*121*). At the present time, beryllium is defined as a strategic metal and is under strict priority regulation (*129*).

The Uses of Beryllium

The alloys of beryllium and copper represent its most important use at the present time (*105*). These alloys are ductile and capable of being cold formed into various shapes and after suitable heat treatment become very hard and have an astonishing fatigue resistance. Springs made of this alloy are said to retain their elasticity almost indefinitely even in atmospheres that are highly corrosive to steel springs. Beryllium copper is used in the diaphragms of altimeters and in gasoline and oil pipe lines in airplanes where vibration is excessive.

The electrical conductivity of beryllium copper is remarkably high, being twice that of steel and rising to 70 percent of that of copper for some alloys. Even in the case of the alloy of highest tensile strength, the electrical conductivity may be 30 percent of that of copper.

The alloy with aluminum having a high beryllium content is lighter than aluminum itself and as strong as steel. As little as 0.005 percent beryllium added to magnesium base alloys containing 8 percent of aluminum is said to decrease the grain size, retard oxidation in melting, and increase resistance to salt water corrosion.

Certain alloys of beryllium and nickel are of importance, and the tensile strength of an alloy of copper, nickel, and 2.25 percent beryllium is more than 200,000 pounds per square inch (*94*).

Owing to its high transparency for X-rays, it can be used as a filter for stray or reflected electrons, and when used as a target for high voltage deuterons produces neutron beams of great intensity (*19*, *50*, *120*). A new type of beryllium glass is especially useful in

X-ray tubes as a window to filter out stray or reflected electrons in X-ray streams. A recent novel use for beryllium is in the production of beryllium alginate for the manufacture of a textile fibre from sea weed (*115*). However, all these minor uses of beryllium are insignificant in comparison with the importance of the fatigue-resistant beryllium copper alloy.

Metallurgy of Beryllium

Brief mention of the more important methods of preparation of metallic beryllium in use at the present time may be of assistance in depicting the potential health hazards of the processes. The only ore of commercial importance employed in the extraction of beryllium is beryl, a beryllium-aluminum silicate, $3BeO \cdot Al_2O_3 \cdot 6SiO_2$. Owing to the refractory nature of this mineral, and its comparative inertness toward acids and alkalies, extraction of beryllium from it is difficult. High temperatures and fusion methods are necessary. The more common processes of extracting beryllium from this ore are as follows:

(*a*) *Fluoride fusion.*—The fusion of crushed beryl with sodium silico fluoride yields beryllium oxyfluoride upon extraction with water. This may be converted into other salts, or used directly in the electrolytic production of beryllium (*76*, *101*, *124*).

At a lower temperature ammonium fluoride alone or in mixture with hydrofluoric acid decomposes beryl. Beryllium fluoride is extracted from the residue and may be subsequently converted to the sulfate.

(*b*) *Alkali fusion.*—In this process crushed beryl is fused at a high temperature with sodium carbonate, or with sodium or potassium hydroxide. The resulting melt is cooled, treated with water, crushed and extracted with hydrochloric acid. Basic beryllium carbonate may be separated from this and, on ignition, yields beryllium oxide.

(*c*) *Sulfuric acid treatment.*—A more recent method than the above involves treatment of beryl with sulfuric acid following prolonged heat treatment of the ore. Beryllium is obtained as the sulfate.

The final step in the production of metallic beryllium consists in electrolysis of molten beryllium halides in a melt of alkali or alkaline earth halides. Beryllium chloride, beryllium fluoride, and beryllium oxyfluoride are the salts most commonly used in the electrolytic process.

Since the alloy with copper is of more importance at the present time, the amount of pure beryllium produced is small. This alloy may be prepared in the electric furnace by direct reduction of beryllium oxide in the presence of copper. The cost per pound of con-

tained beryllium produced in this manner is lower than the cost of production of the pure metal and most of the domestic beryllium is produced in the United States by this means at the present time (*122*).

Exposure to Beryllium Dust and Fume

According to Zamakhovskaya (*133*), the extraction of beryllium by fluoride fusion causes a fume concentration of from 0.05 to 0.72 mg. of beryllium and 1 to 23 mg. of fluorine per $m.^3$ of air in the working environment. While beryllium fluoride dust is given off for the greater part in this operation, in the final stage of production of metallic beryllium it has been stated that fumes of metallic beryllium as well as beryllium fluoride and other fluorides are evolved. These dusts have a high degree of dispersion and, according to Gelman (*46*, *47*), are sufficiently toxic to cause severe poisoning of exposed workers in beryllium foundries.

The mode of entry of beryllium into the system would seem to be chiefly through the respiratory tract rather than through the alimentary tract, but it is probable that some beryllium enters the digestive system, through introduction into the mouth by workmen eating with dirty hands, smoking at work, or chewing tobacco. It has also been pointed out previously (*39*) that a considerable portion of the dust inhaled by mouth or nose is retained in the mouth and throat, to be subsequently swallowed.

The chances for exposure to beryllium dusts are fairly numerous in the industry, and may be encountered during practically all stages of beryllium production. After mining, the ore must be finely crushed before processing, thus workmen are inevitably exposed to beryl dust. The same is true in the ceramics industry where beryl is used to a limited extent.

In the decomposition of beryl, beryllium compounds such as the fluoride, oxyfluoride, sulfate, carbonate, and oxide are produced. Exposure to the dusts of these compounds is almost unavoidable since all are eventually obtained in a dry form. A fairly large quantity of the oxide is used both in the production of beryllium itself and as a super refractory. Next to beryl, the oxide is probably most commonly encountered by workmen.

The oxyfluoride and the chloride are produced in considerable volume, for use in the electrolytic production of beryllium. On the other hand the production and packaging of beryllium compounds for the chemical market is so small that exposure from this source is insignificant.

In view of the reputedly poisonous properties of beryllium, the very rapid growth of the beryllium industry and, despite the relatively small number of workers engaged in the extraction of this metal, it was

considered advisable to investigate the toxicity of beryllium in order to help define the proper hygienic environment of beryllium workers.

II. PHYSIOLOGICAL ACTION OF BERYLLIUM

The degree of physiological response to beryllium compounds has not apparently been sufficiently interesting to attract a great deal of experimental investigation. Furthermore, much of the experimental data is of a conflicting nature. In this regard, however, it must be noted that the toxic action frequently referred to in industrial operations has not been so definitely related to beryllium *per se* as to certain of its compounds, such as the fluorides.

Sestini in 1888 (*109*) found that beryllium may take the place of magnesium in the growth of plants but is not a complete substitute for the latter in the production of seeds. Gies, 1911 (*49*) found that the growth of lupin seedlings received a slight initial stimulus from solutions of beryllium sulfate but that stronger solutions retarded growth. Similarly Seaman, 1912 (*108*) found that beryllium sulfate had a marked inhibiting effect on the growth of lupin and timothy seed. Bambacioni-Mezzetti, 1934 (*5*) found that beryllium chloride affected the geotropic sensitivity of plants although less than the salts of palladium or zirconium. Lehr, 1926 (*67*) found that beryllium greatly inhibits the urease activity of *B. proteus*. On the other hand, Lepierre, 1913 (*68*) found that beryllium could be assimilated and fixed by *Aspergillus niger* and Duncan and Miller, 1936 (*31*) found that the beryllium content of the soil of northern Michigan was not responsible for any toxic effect. Mazé and Mazé (*82*) have found that small amounts of beryllium chloride have a beneficial action on the growth of plants.

Much more evidence of the adverse effect of beryllium has been reported on animals than upon plants. A certain amount of the early work with reference to the toxicity of beryllium is so inconclusive, however, that it would appear that some of these early observations were erroneous. As early as 1882 Blake (8, 9) asserted that the general physiological effects of beryllium were similar to those of aluminum and iron. Brunton and Cash, 1884 (*13*) found beryllium chloride to be no more toxic to frogs than potassium chloride or calcium chloride.

In a study of the comparative toxicity of aluminum and beryllium, Siem, 1885 (*111*) found that the subcutaneous and intravenous injections of beryllium were more toxic than those of aluminum. He used solutions of the lactate and tartrate salts of beryllium, calculated his doses in terms of beryllium oxide and stated that from 0.02–0.028 gm. of beryllium oxide was a lethal dose for a medium sized frog; from 0.004–0.005 gm. beryllium oxide per kilo of body weight for dogs and

cats; and from 0.008–0.01 gm. per kg. of body weight for rabbits. Kunkel (*66*) commenting on Siem's results points out the uncertainty with which manifestations of toxicity appear following the administration of beryllium compounds.

It is interesting to note how variously experimental results have been interpreted. Thus, Mines, 1910 (*88*) studying the action of beryllium, lanthanum, yttrium, and cerium on the frog's heart concluded that beryllium solutions have a powerful action on the frog's heart. However, Mines states that this is due to hydrolysis and consequent acidity of the salt solution.

On the other hand, Seaman, 1912 (*108*) stated that beryllium sulfate has a marked effect on biochemical processes. When administered with the food it produced decided nutritive disturbance which manifested itself in loss of body weight, as well as metabolic disturbances with reference to total inorganic matter, nitrogen, sulfur, and phosphorus.

Furthermore, in 1928, Duliére and De Borggraef (*30*) noted the effect of beryllium on the irritability of frog's heart and stated that when small amounts of beryllium replaced calcium or potassium in Ringer's solution, an increased irritability resulted.

The lethal dose for beryllium on subcutaneous injection was determined by Richter in 1930 (*99*). She investigated the effects of beryllium nitrate on blood serum, blood corpuscles, yeast, frog's heart, and paramecium, and found the lethal dose of beryllium nitrate when subcutaneously injected into white mice to be 50 mg. per kg. of body weight.

Similarly, Wunderlich, 1934 (*132*) in his experiments showed that subcutaneous injections made every second day of 1 to 2 ml. of a 3-percent solution of beryllium chloride to rats, or nitrate to guinea pigs was injurious and in the end, fatal.

The pathological changes associated with beryllium poisoning were noted by Comar, 1935 (*20*) who found that the liver in acute intoxication, either with a single dose or with repeated doses of beryllium sulfate, showed an increase of volume with diffused hepatitis. Furthermore, the kidneys presented the lesions of diffused nephritis.

Further investigation was made by Lorenz, 1936 (*72*) who studied the resorption, distribution, and excretion of beryllium in warm-blooded animals. After subcutaneous injection of beryllium nitrate, beryllium could be detected in the urine of rats even after several hours. Toward the end of the second day, the beryllium excretion in the urine was small. Beryllium was present in the intestinal contents even on the fourth day. Beryllium was found in the liver, brain, blood, and striated musculature.

An interesting review was made in 1937 by Steidle (*116*) who described the work of Richter with reference to the action of beryllium chloride and beryllium nitrate on serum, fermentation of yeast, isolated frogs' hearts and vessels, red corpuscles, uterus, eye, and mucous membranes and also its effect on paramecia, fish, frogs, rats, and cats. Steidle has related its effect to that of magnesium on the one hand and aluminum and the rare earths on the other.

The effect of beryllium intoxication on blood sugar has recently been a matter of investigation. Sutton in 1939 (*119*) observed that acute intoxication by beryllium caused an increase in the hemoglobin concentration and number of red blood corpuscles and a fall in the blood pressure. The blood sugar concentration, at the same time, was increased following acute intoxication. De Conciliis in 1939 (*25*) found that when beryllium oxide was injected subcutaneously into rabbits a definite effect was indicated by the changes in the glycemic curve.

Other changes were observed by Caccuri, 1940 (*16*) who found that animals injected with beryllium compounds showed hepato-renal changes. This investigator considers beryllium to be a toxic substance capable of producing a hepatonephritis.

More recently, Volter, 1940 (*125*) has found that beryllium as the chloride or fluoride is similar in toxic properties to that of heavy metals. It is, however, slower in action, and the fluoride was found to be more toxic than the chloride.

Beryllium Rickets

An interesting phase of the various investigations with reference to the action of beryllium is that related to so-called beryllium rickets. This comparatively recently described condition was first indicated by Branion, Guyatt, and Kay, 1931 (*11*) who found that by replacing the calcium carbonate in Steenbock's rachitogenic ration with beryllium carbonate, bone lesions similar to rickets may be easily produced in young rats. Kay, 1932 (*59*) and Kay and Guyatt, 1933 (*60*) found that with beryllium rickets there is a marked diminution in the phosphoric ester content of the red blood cells. Kay and Skill, 1934 (*61*) showed that beryllium rickets can be prevented by the simultaneous parenteral administration of the sodium salt of β-glycerine phosphoric acid. From this the authors concluded that beryllium rickets is caused by deficient absorption of phosphates from the intestine.

Jacobsen, 1933 (*57*) fed growing rats with calcium-deficient food and the addition of cod liver oil. Osteoporosis occurred, while with the addition of beryllium carbonate, rickets developed.

The work of Fabroni (1933–35) (*34*, *35*, *36*, *37*) is of interest in this connection. Fabroni found that the feeding of small daily doses

of pure beryllium salts (chloride, colloidal hydrate, and iodide) to growing guinea pigs produced a progressive increase in body weight over that of the controls. After feeding 1 to 2 percent basic beryllium carbonate to guinea pigs in food, Fabroni could find no skeletal deformities, decalcification of the epiphyses nor fragility of the bones though he radiographed the animals periodically and after their death made a histological examination of their bones. He attributed the osteological findings obtained by Guyatt, Kay, and Branion, 1933 (*52*) to possible traces of fluorides in the basic beryllium carbonate.

In 1935, Sobel, Goldfarb, and Kramer (*114*) produced rickets in young rats by feeding small amounts of beryllium carbonate.

However, Loomis and Bogen, 1935 (*71*) in an investigation of the chemotherapy of experimental tuberculosis in guinea pigs using beryllium chloride, found that this salt in doses of 1 to 5 mg. administered to normal animals had little effect on their general health, weight curve, or blood count. The beryllium rickets reported in rats on diets containing large amounts of beryllium was not observed in these guinea pigs.

Some investigation of beryllium rickets from a more remote source than the soluble compounds noted above, has been made by Duncan and Miller, 1936 (*31*). These investigators found that the soil of northern Michigan contained 0.615 percent beryllium oxide which was equivalent to 0.223 percent beryllium. The beryllium occurred there as beryl. The feeding of a diet which contained 30 percent soil to rats did not predispose them to any of the rachitic symptoms produced in rats which received 0.25 percent beryllium carbonate in their diet.

It is of interest that Jones, 1938 (*58*) found aluminum, beryllium, and strontium each produced marked rickets in rats with accompanying low serum phosphate.

Similarly, Businco, 1939 (*14*, *15*) induced rickets in young rats in from 15 to 40 days on a diet containing beryllium carbonate and which he demonstrated by both X-ray and histological examination. This investigator believes that beryllium rickets cannot be a deficiency manifestation, but must be attributed to a definitely toxic action of beryllium.

Berylliosis

Fabroni has coined the term "berylliosis" for the type of lung damage caused by inhalation of the dust or fumes of beryllium compounds. This condition was first referred to in 1933, by Weber and Engelhardt (*130*), who made an experimental study of the effects on guinea pigs of breathing the dusts encountered in the commercial production of beryllium. Their experiments indicated that the type of damage produced by various beryllium compounds on inhalation was similar to that produced by such corrosive gases as phosgene or

chlorine. The dusts used were sodium silico-fluoride and a combination of sodium aluminum fluoride and silicic acid. These experiments indicate that the cause of the diseases in beryllium plants is due to the fluorides occurring there in the form of dust—a point with which Fabroni takes issue since he regards beryllium carbonate itself a causative agent. The researches of Zamakhovskaya, 1934 (*133*) and the clinical observations of Martsinskovsky and Syroechkovsky, 1934 (*77*) reported by Gelman (*46*, *47*), indicate that the toxicity is due to the beryllium fluorides of which beryllium oxyfluoride ($2BeO{\cdot}5BeF_2$) is the principal toxic agent. Gelman furthermore emphasizes the severe poisoning of exposed workers which occurs in beryllium reduction plants. Berkovits and Israel, 1940 (*7*) conclude that the changes in the lungs in beryllium fluoride poisoning are specific and that in the average cases X-ray studies show emphysema and increase in connective tissue with lymphocytic infiltration. Fabroni, 1935 (*36*) concludes that the type of lung damage produced by the inhalation of beryllium dusts is typical for beryllium itself, while Menesini, 1937 (*84*) has concluded that beryllium causes systemic poisoning, rather than a pneumoconiosis only.

Therapeutic Use of Beryllium

Beryllium is but one of the many metals whose salts have been exploited as having curative properties and a certain number of therapeutic claims have arisen at various times.

Walbum, 1925–26 (*127*, *128*) and Lunde, 1926 (*74*), Gessner and Siebert, 1930 (*48*) using minimal doses of beryllium salts for injection of experimental animals infected with tubercle bacilli found an increase in defense power of the animals especially toward intoxications caused by mass infections. These claims could not be substantiated by Wunderlich, 1934 (*132*). The chemotherapeutic use of beryllium and its compounds has been further explored by Purdy and Walbum, 1922 (*98*), Fabroni, 1933 (*34*, *35*, *37*), Cuneo, 1935 (*23*), Loomis and Bogen, 1935 (*71*), Lorenzoni, 1938 (*73*), and Parlavecchio, 1939 (*96*). None of the evidence appears to be sufficiently decisive, nor adequately authenticated at the present time to carry much weight with reference to the therapeutic use of beryllium.

III. THE ANALYTICAL DETERMINATION OF BERYLLIUM

Earlier Procedure

The analytical literature of beryllium has been thoroughly reviewed by Parsons, 1908 (*97*) and by Mellor, 1923 (*83*). Fischer, 1926 (*41*) reviewed the literature on the analysis of beryllium to 1926. Since 1926 there have been numerous reports (*18*, *26*, *27*, *62*, *64*, *75*, *89*, *90*, *92*, *100*, *136*) on the separation of beryllium from other metals, and

the determination of the separated beryllium. However, the final estimation of beryllium, especially in small amounts, has usually proved to be difficult. Gravimetric (*1*, *2*, *24*, *42*, *44*, *45*, *55*, *91*, *107*), volumetric (*33*, *135*), and colorimetric (*41*, *43*, *63*, *85*, *112*) procedures, fluorescence analysis (*6*, *28*, *51*, *103*, *104*, *131*, *134*) and microchemical analysis (*17*, *65*) have all been proposed. These various methods were examined or applied to the determination of beryllium in animal tissues in this laboratory, but were generally found unsuitable for the very small amounts of beryllium involved in the present investigation. After reviewing and testing such of these procedures as appeared to be applicable, none was found to be sufficiently accurate for use in the present investigation, and it was found necessary to evolve special methods for the determination of beryllium in animal tissues.

Newer Methods for Determination of Minute Amounts of Beryllium

After investigating the various methods currently reported for the determination of beryllium and after extended experimental work had been done to establish a satisfactory procedure, three possible tests were finally applied and found to give satisfactory results. One of these was colorimetric in character; both the other two involve fluorescence as a means of detection. The conditions under which they were most useful were found to be different and all three were therefore employed in the final investigations.

Colorimetric method.—The commonly advocated curcumin and quinalizarin (*56*) methods were found to be unsatisfactory for the quantitative estimation of beryllium since insufficient differences in color were given with the minute quantities of beryllium in the tissues of exposed animals. However, in the course of the experimental work an entirely new reagent was found to give a distinctive test for beryllium. In investigating various anthraquinone derivatives as color reagents for beryllium, 1,4-dihydroxyanthaquinone-2-sulfonic acid (quinizarin, 2-sulfonic acid), buffered at pH 7.0 with ammonium acetate, was found to give a red color which is proportional to the amount of beryllium. The color develops rapidly, reaches a maximum in five minutes, and does not fade for several hours. The most satisfactory range for colorimetric comparison in a visual colorimeter is 1 to 10 μgm. of beryllium.

Fluorescence method.—The reagent 1-amino-4-hydroxyanthraquinone[1] proposed by White and Lowe (*131*) was also applied to the determination of beryllium in animal tissues. This reagent produces a red fluorescence when the solution is made slightly alkaline. The

[1] Obtainable from the National Aniline Division of Allied Chemical and Dye Corporation, New York, New York.

fluorescence is proportional to the amount of beryllium in the range from 0.05 to 10 μgm. when the test solutions are compared visually in ultraviolet light. A simple fluorescimeter (fig. 1) was constructed for this purpose. A small quartz mercury arc lamp of the A–H3 type in conjunction with a Corning glass filter No. 585 (8 mm. thick) is convenient for use in this apparatus.

Another reagent, 1,4-dihydroxyanthraquinone, produces a red to yellow fluorescence with beryllium under identical conditions. Fluorescence with either 1-amino-4-hydroxyanthraquinone or with 1,4-dihydroxyanthraquinone occurs in acid or neutral solution due to the

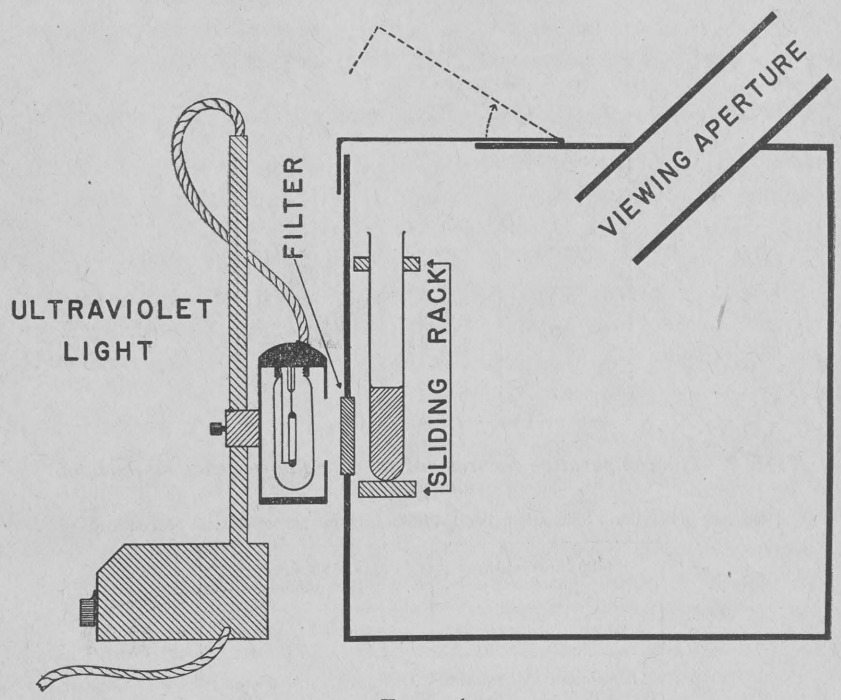

FIGURE 1.

A visual comparison fluorescimeter.

dye itself. In alkaline solution the fluorescence due to the dye disappears but with the addition of beryllium strong fluorescence is apparent.

Comparison was made of these three methods in the analysis of fresh lung tissue of animals that had been exposed to beryllium carbonate dust by inhalation. The results are given in table 1. Considering the small amounts of beryllium present, the three methods give values sufficiently accurate for the small amounts of beryllium to be evaluated in the various animal tissues.

A comparison of the two fluorescence methods of beryllium analysis was made by adding known amounts of beryllium to beryllium-free solutions of ashed liver. The values obtained by this procedure are shown in table 2, and compare favorably considering the small amounts of beryllium involved.

Table 1.—*Comparison of the results obtained by the colorimetric and fluorescence methols of analysis*

[Milligrams beryllium found in 10 gm. fresh lung tissue from animals exposed to beryllium dust]

Fluorescence method		Colorimetric method
1-amino-4-hydroxy-anthraquinone	1,4-dihydroxy-anthraquinone	1,4-dihydroxy-anthraquinone-2-sulfonic acid
Mg. per 10 gm.	*Mg. per 10 gm.*	*Mg. per 10 gm.*
1.0	1.0	0.9
2.0	2.0	1.9
4.0	5.0	4.6
4.0	3.0	3.6
4.0	4.0	4.1
2.0	2.0	3.0
4.0	4.0	3.5
4.0	3.0	3.4
2.0	3.0	2.5
3.0	3.0	2.7
3.0	3.0	3.4
3.0	3.0	2.5
12.0	12.0	9.1
8.0	8.0	7.9
9.0	9.0	8.3
10.0	10.0	8.0
Av. 4.7	Av. 4.7	Av. 4.3

Table 2.—*The comparative accuracy of the two fluorescence methods of analysis*

[Recovery of beryllium (micrograms) added in known amount to solutions of liver ash]

1, 4-dihydroxyanthraquinone		1-amino-4-hydroxyanthraquinone	
Added	Found	Added	Found
Micrograms	*Micrograms*	*Micrograms*	*Micrograms*
5.0	5.0	4.0	5.0
8.0	9.0	9.0	9.0
7.0	8.0	0	0
10.0	10.0	0.5	0.5
4.0	4.0	2.0	1.5
3.0	3.0	7.0	7.0
1.5	1.5	1.5	2.0
9.0	9.0	3.0	3.0
6.0	7.0	4.0	4.0
1.0	1.0	10.0	10.0
1.0	0.5	0	0
0.5	0.5	1.5	1.5
10.0	10.0	3.0	3.0
8.0	9.0	4.0	5.0
6.0	6.0	2.0	2.0
Av. 5.3	Av. 5.6	Av. 3.4	Av. 3.6

In general, blank determinations were made with normal tissues similar to those under investigation. In order to rule out the possi-

bility of the adverse effects of certain of the organic tissue constituents, the animal tissues analyzed were previously sampled, dried, ashed in an electric muffle furnace, dissolved in 1 N hydrochloric acid, and, in the case of soft tissue, made up so that 2 ml. of solution would equal one gram of tissue.

Spectrographic examination of bone ash for beryllium.—Beryllium gives a number of well defined and characteristic lines in both the spark and arc spectra and various workers have reported methods for the quantitative determination of beryllium by spectrographic methods (*78*, *102*, *118*).

Although no attempt was made to determine the beryllium quantitatively, a series of qualitative arc spectra were run on bone ash solutions, obtained from guinea pigs which had ingested or inhaled beryllium compounds.[2] The beryllium doublet at 3321.35–3321.08Å and the persistent line of 2348.61Å were used to identify beryllium. Chemical tests for beryllium were positive in all those cases where the spectra showed beryllium to be present, and it was also noted that the lines in the spectra were strongest where chemical analysis indicated the largest amount of beryllium.

Procedure for Determination of Beryllium in Dust, Tissues, Blood, Urine, and Feces

1. *Colorimetric determination.*—The weakly acid solution of beryllium dust, or the ash of urine or feces is diluted to a convenient volume. The phosphates, if present, are removed by zirconium nitrate and the resulting excess of zirconium removed by selenious acid. To 1 ml. of this solution containing beryllium, after adjustment of the hydrogen ion concentration to a pH of 3.5, add 5 ml. of 5 percent ammonium acetate solution and 0.2 ml. of a 0.5 percent aqueous solution of 1,4–dihydroxyanthraquinone–2–sulfonic acid. Prepare a set of standards with known amounts of beryllium ranging from 0–10 μ gm. of beryllium. After standing at least 5 minutes, the tissue sample and standards may be compared in a visual colorimeter.

2. *Fluorescence analysis.*—Pipette 0.5 or 1 ml. of the tissue ash solution into a pyrex test tube. Add an equal volume of 10 percent sodium citrate solution and make up to a volume of 4 ml. with distilled water. Add 0.2 ml. of a 0.03 percent solution of either 1-amino-4-hydroxyanthraquinone or 1,4-dihydroxyanthraquinone in 95 percent alcohol. Add 2 N sodium hydroxide with shaking until the solution changes in color from red to violet. An additional 2 drops gives a sodium hydroxide concentration of about 0.05 N. Compare

[2] The authors are indebted to Associate Physicist D. W. Armstrong of this laboratory for his kindness in making these tests.

this solution in filtered ultraviolet light with a set of standards similarly prepared but containing known amounts of beryllium.

The quantitative determination of beryllium in bones is difficult in comparison with its determination in other tissues and the procedure finally adopted is at best a method of approximation only. In the final evaluation, it was necessary to correct the values obtained, by comparison with the recoveries of known amounts of beryllium added to beryllium-free bone tissue, similarly treated. To the hydrochloric acid solution of the ashed bone tissue, add an excess of sodium hydroxide in order to precipitate the calcium and magnesium phosphates and in sufficient excess to retain the beryllium in solution. Boil, filter, acidify the filtrate with hydrochloric acid, neutralize, make slightly alkaline with ammonium hydroxide and let stand overnight. The precipitated beryllium hydroxide is treated with hydrofluoric acid to free it from silica, dissolved in hydrochloric acid, and tested for beryllium by the fluorescence method. Since the recovery of beryllium by this method is not as quantitative as that of soft tissues it was necessary to correct the values obtained. This was accomplished by comparison with values obtained by a similar treatment of bone ash solutions to which known amounts of beryllium had been added.

In analyzing blood for its beryllium content, it is ashed in an electric muffle furnace at about 600° C. The hydrochloric acid solution of the ash corresponding to 5 gm. of blood is treated with ammonium hydroxide, the gelatinous precipitate separated, dissolved in 2 N hydrochloric acid, treated with freshly prepared 5 percent cupferron solution, the iron cupferron complex extracted with chloroform, and the residue analyzed by the fluorescence method.

No one of these methods was found to be generally applicable. Their use depended upon the type of biological material under examination. It was found, for instance, that the colorimetric method was best suited to the analysis of samples of dust taken from the exposure chamber and for urine and feces, while the fluorescence methods were best suited for the analysis of soft tissues, blood, and bones.

IV. EXPERIMENTAL PROCEDURE AND APPARATUS

The following compounds of beryllium were investigated by means of animal experimentation in order to determine their toxicity when administered by mouth, intraperitoneal injection, and by inhalation of dust and fumes: beryllium oxide, carbonate, phosphate, chloride, sulfate, nitrate, oxyfluoride, hydroxide, potassium beryllium sulfate, and the mineral beryl (beryllium aluminum silicate). The means devised for this purpose varied somewhat from those of the previous investigations in this laboratory owing to the somewhat unusual properties of beryllium compounds.

Guinea pigs, white rats, white mice, rabbits, and dogs were used as experimental animals. The weights of the animals were recorded weekly throughout the test.[3] Hemoglobin estimations, erythrocyte counts and blood smears for the study of blood morphology were made on many of the animals. At autopsy, the gross pathological changes were noted and portions of the organs were sectioned and examined microscopically for pathological changes. The lungs, kidneys, liver, and skeleton were analyzed for beryllium content.

Intraperitoneal Injection

Sterile solutions or suspensions of beryllium compounds in physiologic saline solution were injected intraperitoneally into guinea pigs and mice, following the technique of Miller and Sayers (*87*), to determine the relative toxicity of the various compounds. Animals were also injected with beryllium, magnesium, and zinc compounds in an attempt to find the comparative toxicity of these three elements. The animals were sacrificed after a sufficient length of time and the tissues were removed for chemical analysis and microscopic examination.

Oral Administration

Rats weighing 130 to 185 gm. were placed five in a cage and fed beryllium compounds (beryllium content ranging from 5 to 50 mg. per day) for a period of 4 months. The beryllium compounds were made into solutions or suspensions and a measured amount added to the rat diet (*40*). The diet, composed of wheat flour, corn meal, oat meal, yeast, dextrin, powdered milk, and liver powder with the addition of adequate mineral salts, was fed in the form of a stiff dough.

Guinea pigs were fed varying amounts of beryllium phosphate, carbonate, and sulfate. A measured amount of beryllium solution or suspension was poured over the weighed daily ration of commercial guinea pig food in the form of pellets, and dried. The diet was supplemented daily by greenstuff. The feeding was continued for from 3 to 6½ months.

Inhalation of Dusts

A series of experiments was carried out in which guinea pigs were exposed to dusts of beryllium compounds. The compounds used included: beryllium carbonate, beryl, anhydrous beryllium sulfate, anhydrous beryllium oxyfluoride, and a hydrated potassium beryllium sulfate ($K_2Be(SO_4)_2 2H_2O$). Exposure periods amounted to 40 minutes per day in the majority of the cases but in a few instances were limited to 30 minutes per day. The animals were exposed in groups of from 6 to 16 in a chamber of the type shown in fig. 2. The chamber, a modification of the one designed by Dudley (*29*), measured

[3] We are indebted to Under Scientific Aide Leonard A. Fulton for technical assistance.

64 cu. ft. in volume, was constructed of masonite, wood, and glass, and was reasonably airtight.

The elutriating apparatus (*39*) yielded finely particulate dust of uniform size at a steady rate. Compressed air, measured with a flow meter, was led into the glass container holding the beryllium compound through a cone-shaped apparatus. From the bottom of this cone four very small tubes carried the air through the finely ground beryllium compound. A cyclonic effect was produced and the dust was carried up into an elutriating cylinder, from which it passed into the exposure chamber. In the case of beryllium oxyfluoride, it was necessary to dry the air by means of a calcium chloride tube, because of the hygroscopic nature of the compound. Even with this, it was difficult to obtain a dusty atmosphere.

The air in the exposure chamber was sampled by collecting the dust from a measured volume of air (*39*). Air samples of a volume of 0.5 cu. ft., as measured by a meter, were drawn through filter paper discs and the latter analyzed for beryllium. An Owens dust counter was also used to sample the beryllium carbonate and beryl dusts. The samples thus obtained were mounted on a slide, and the particle size determined by measurement with a filar micrometer. Size frequency curves, median values, and arithmetic deviations were calculated from the data obtained.

Exposure to Fumes Produced During the Electrolytic Deposition of Beryllium

Since various workers have reported toxic effects caused by the inhalation of fumes liberated during the electrolysis of beryllium salts, it was decided to ascertain, if possible, what part beryllium itself plays in such toxicity.

The animals were exposed to the fumes resulting from electrolysis in an exposure chamber of the type shown in figure 3, and having a volume of 8 cu. ft. The front of the chamber was made of heavy glass, fitted against a soft rubber gasket and was easily removable.

The electrolysis was carried out in a graphite crucible approximately 5 cm. in diameter and 7.5 cm. deep. The crucible served as the anode, while the cathode was an iron rod, 1 cm. in diameter, arranged so as to be easily raised and lowered. The whole was housed in an improvised glass hood.

Air was drawn through the hood into the chamber by means of a vacuum pump, the volume of air being measured with a flow meter. An absorption train, consisting of five wash bottles partly filled with water, was used to remove soluble and insoluble matter from the air as it was drawn from the chamber.

The electric current was drawn from a 120-volt D. C. circuit having a capacity of 50 amperes. A resistance unit, consisting of five

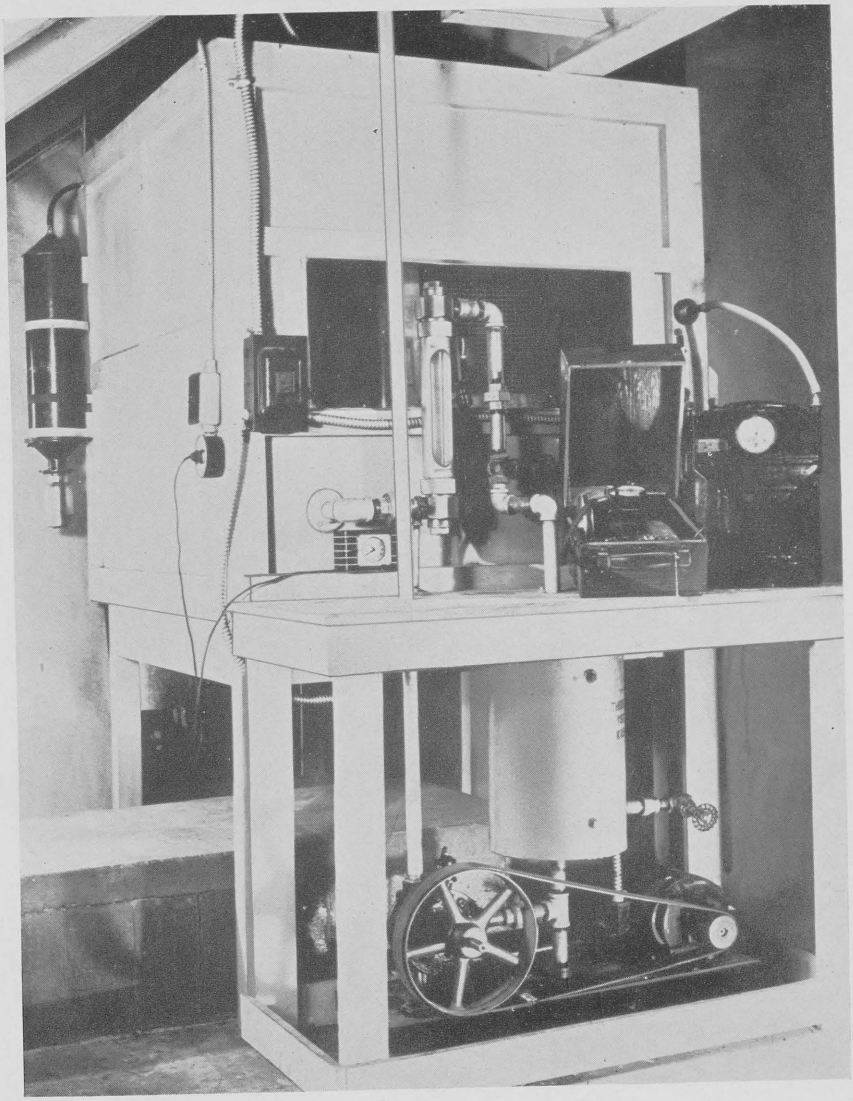

FIGURE 2.

The exposure chamber and elutriator used for exposing animals to beryllium dusts.

1000-watt heating coils connected in parallel, was placed in the circuit, as shown in fig. 3.

In the selection of a method from the several which have been used industrially, the Stock-Goldschmidt process was chosen (*79*), because it was apparent that the very high temperature conditions under which this process is carried out made it more likely to produce toxic fumes than processes carried out at lower temperatures. In the Stock-Goldschmidt process, beryllium is produced in coherent form by electrolyzing a melt of beryllium oxyfluoride ($2BeO \cdot 5BeF_2$) and barium fluoride at a temperature above the melting point of beryllium, i.e., 1350°–1400° C. An easily fusible mixture of sodium

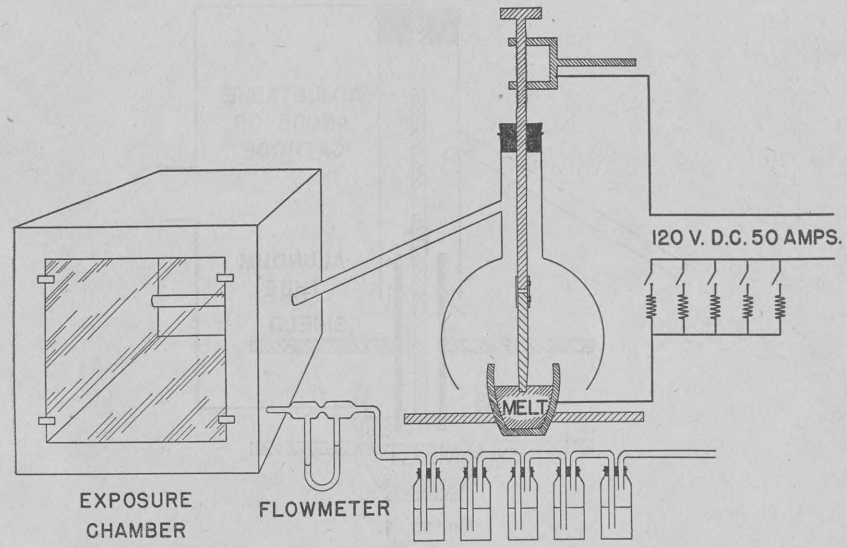

FIGURE 3.

The apparatus for exposing animals to electrolysis fumes.

fluoride and beryllium oxyfluoride is used to start the electrolysis, but once a high temperature is attained, the melt is continuously replenished with a beryllium oxyfluoride-barium fluoride mixture, in which the barium fluoride content varies from 50 percent upward.

To start the electrolysis, a small amount of sodium fluoride and beryllium oxyfluoride mixture was placed in the crucible and fused by means of a gas flame. When a melt was obtained, the cathode was lowered to start the electrolysis. A current of about 80 volts and 40 amperes was passed through the melt, resulting in a rapid rise in the temperature. A mixture of 50 percent barium fluoride (BaF_2) and 50 percent beryllium oxyfluoride ($2BeO \cdot 5BeF_2$) was then added until the crucible was about two-thirds filled. The

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cathode was raised so that the tip extended just below the surface of the melt, this being necessary to maintain a high surface temperature.

When the melt was a clear red and the electrolysis running smoothly, the glass hood was lowered so that it surrounded the crucible. Connection was made to the chamber and the air flow so adjusted that 0.25 cu. ft. of air per minute was drawn from the chamber throughout the exposure. The animals were then placed in the chamber and the exposure commenced. Exposure times varied

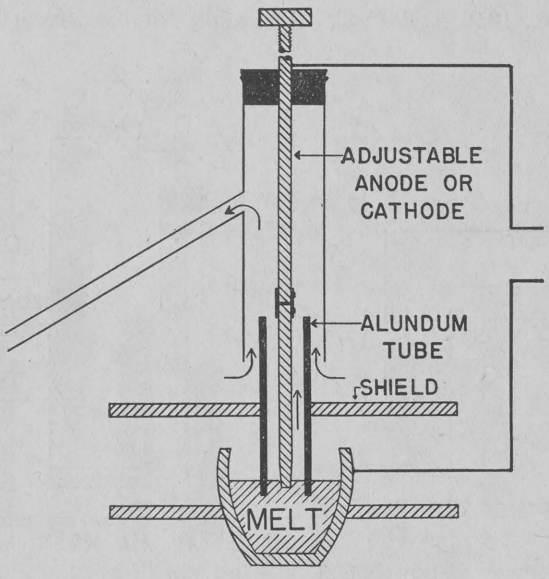

FIGURE 4.

A device for isolating anode and cathode fumes.

from 20 to 150 minutes. Occasionally during the exposures it was necessary to strike an arc between the cathode and the molten surface in order to maintain a high surface temperature, or, in lieu of this a flame was sometimes kept under the crucible. Neither operation seemed to affect the toxicity of the fumes. The temperature in the exposure chamber varied from 28° to 31°C.

Experiments were also made in which the gaseous products of the anode and the cathode, respectively, were isolated and animals exposed solely to one or the other. The apparatus used for this purpose is indicated in figure 4. Both rats and guinea pigs were used as experimental animals.

V. EXPERIMENTAL RESULTS

Intraperitoneal Injection of Beryllium Compounds

As indicated above, similar quantities of beryllium salts or compounds either in solution or suspension were intraperitoneally injected into guinea pigs. The mortalities of these animals at intervals of 4 days, 4 weeks, and 4 months are shown in table 3. The mortality of the guinea pigs for 4 weeks is also shown in figure 5.

At a level of 0.1 gm. of beryllium compound injected in guinea pigs averaging 578 gm. in weight, a mortality of 100 percent at the end of 4 days was noted in all cases where the beryllium compound

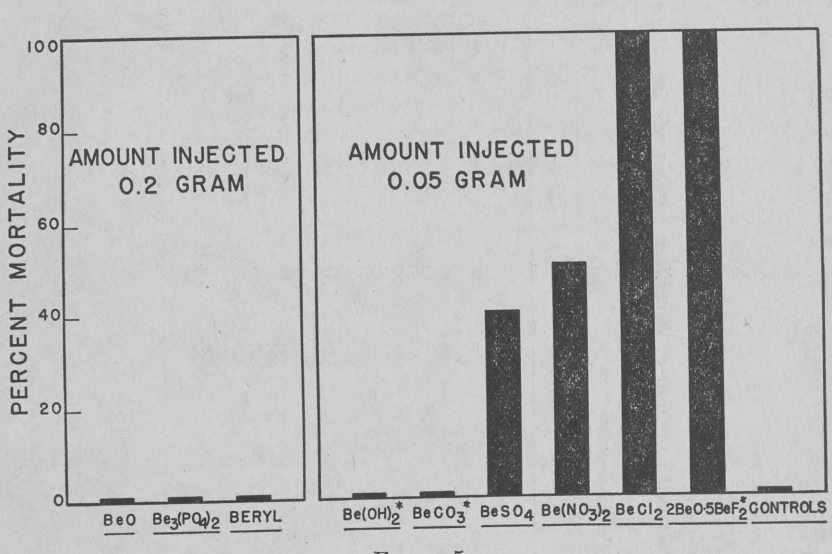

FIGURE 5.

The mortality of guinea pigs in 4 weeks following intraperitoneal injection of various amounts of beryllium salts.

was soluble (beryllium chloride, nitrate, sulfate, and oxyfluoride). The mortality in reference to beryllium oxyfluoride was striking, for, even with 0.005 gm. injected, the mortality was 100 percent. With the more insoluble compounds, no striking mortality was observable at these concentrations even after 4 months. In this connection it might be recalled that beryllium is the least basic of the alkaline earth metals and that the salts such as the chloride and nitrate hydrolyze readily, while an aqueous solution of beryllium sulfate is sufficiently acid to dissolve zinc with the evolution of hydrogen. It is, therefore, highly questionable whether the toxicity as determined in this way represents the toxicity of the beryllium per se, as much as the corrosive action of the liberated acid. This is further borne out by the fact that

Table 3.—*Intraperitoneal injection of beryllium compounds in guinea pigs*

Compound injected	Amount injected	Animals injected	Percent mortality			Time on test	Mg. Be/10 gm. tissue				Hemoglobin	Erythrocytes
			4 days	4 weeks	4 months		Liver	Lung	Kidney	Bone		
	Grams	*Number*	*Percent*	*Percent*	*Percent*	*Weeks*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Grams*	*Millions per mm.*^{3}
Beryllium oxide	0.2	8	0	0	0	35	0.206	0.008	0.006	0.019	13.4	5.2
Beryllium carbonate	0.2	7	0	100	100	1						
Do	0.15	2	50	50	50	19	0	0				
Do	0.08	3	0	0	0	19	0.053		0.005			
Do	0.04	4	0	0	25	26	0.037	0	0.	0.062	13.3	5.4
Beryl	0.2	8	0	0	0	35	0.018	0	0.008	0.064	13.9	5.3
Beryllium phosphate	0.2	4	0	0	0	26	0.020	0	0.010	0.040	13.4	5.6
Beryllium hydroxide	0.04	3	0	0	0	25	0.007	0	0.027	0.058	13.3	5.7
Beryllium chloride	0.05	4	100	100	100	<1						
Do	0.025	3	0	0	0	25	0	0	0	0.035	12.6	5.4
Beryllium sulfate	0.2	4	100	100	100	$<<1$						
Do	0.1	4	100	100	100	<1						
Do	0.05	5	0	40	60	25	0	0.05	0	0		5.5
Beryllium nitrate	0.1	2	100	100	100	<1						
Do	0.05	2	50	50	50	20						
Beryllium oxyfluoride	0.2	4	100	100	100	$<<1$						
Do	0.02	4	100	100	100	<1						
Do	0.005	6	83	100	100	0.14	0.06	0.02	0.01			
Control		6	0	0	17	35	0	0	0	0	13.0	5.1

there were survivors even at the end of 8 months among those receiving the more insoluble compounds.

At the end of 34 weeks the average weight of the control guinea pigs was 931 grams, while those injected with beryl and beryllium oxide weighed 908 and 882 gm., respectively. Animals injected with the carbonate, chloride, hydroxide, and phosphate gained weight less rapidly even though the amount injected was less than in the case of beryl and beryllium oxide. During the 26 weeks following injection, their gain in weight was from 100 to 200 gm. less than that of the controls. The animals, however, appeared to be in good health throughout the test.

The injected animals were sacrificed at the end of the experiment. The abdominal wall and parts of the liver, lungs, kidneys, spleen, and heart were sectioned and examined for pathological changes. The degree to which beryllium was present in the tissues was determined by chemical examination. The beryllium content of the liver, lungs, kidneys, and bones (mg. beryllium per 10 gm. fresh tissue) is shown in table 3. At autopsy 6 months after injection no beryllium was found in the livers of the pigs injected with the soluble salts, chloride and sulfate, while in the case of the insoluble compounds, beryllium oxide, phosphate and beryl, the walled off material could often be seen on the liver as well as on the belly wall of the pigs. This is discussed in the section on pathology.

The beryllium present in the skeleton would indicate that the "insoluble" salts are somewhat soluble under body conditions and that distribution can occur through the medium of the blood stream if given sufficient time. At the termination of the experiment the erythrocyte counts ranged from 5.2 to 5.7 millions per $mm.^3$ with a value of 5.1 millions for the control animals. The hemoglobin (Newcomer method) under similar conditions varied from 12.6 to 13.9 gm. with a value of 13.0 gm. for the control animals (table 3). There was no evidence therefore of polycythemia nor of diminished hemoglobin. Eight guinea pigs born to beryllium oxide and beryl injected mothers, gained weight at the same rate as a litter of control pigs over a period of 4 months.

Ingestion of Beryllium Compounds

In order to determine the effect of ingested beryllium on animals, a series of ingestion experiments with guinea pigs and white rats was carried out with emphasis placed on the following points:

1. The general effect on the health of the animals, i. e., weight changes, mortality rate, morbidity, blood changes, etc.;

2. The distribution of beryllium in the soft tissues and bone, and the relation of such findings to the general effects produced in the animals, if possible; and

3. The alleged rachitogenic properties of beryllium.

No effort was made to establish the lethal dose for each of the beryllium compounds, but rather, doses smaller than those required to produce immediate effects were fed over a long period of time. By so doing, it was believed that any physiological and pathological changes caused by absorption of beryllium would be more evident than would be the case with doses large enough to produce death in a short time.

General effect on health.—A summary of the entire ingestion series including character and duration of each experiment, comparative weight changes, and tissue distribution of beryllium is given in table 4. Each horizontal column represents average results from four animals in the case of guinea pigs and five animals for the white rats.

In addition to the analyses reported in the above table, analyses were made of the hearts, spleen, and leg muscles of seven representative guinea pigs which had ingested various beryllium salts over an extended period of time. These tissues were all practically free from beryllium. Additionally, 60 analyses were made of blood samples of pigs which had been exposed by inhalation, ingestion, and intraperitoneal injection to various beryllium salts without the indication of more than a remote trace of beryllium in the blood in any instance.

Inspection of the weight change values fails to reveal conclusive evidence that beryllium has a retarding effect on the growth of guinea pigs and white rats. No striking difference appeared to exist between different animal species in this respect although as is shown later (p. 40) the degree of absorption appears to vary from animal to animal. In 14 groups the weight gain of the test animals was less than that of their controls, but the reverse was true for the remaining 7 groups of animals. The average gain for guinea pig and rat controls was 364 and 60 gm., respectively, while for the test animals it was 318 gm. for the guinea pigs and 57 gm. for the rats. Most of this difference occurs in the groups which were fed the soluble beryllium sulfate and, beryllium oxyfluoride, both of which are strongly acid in solution. The average guinea pig weight gains for these groups are 261 gm. and 164 gm. for control and test animals respectively. It is difficult to decide whether these differences in weight gains are attributable to beryllium, or to loss of appetite because of excess acidity of the hydrolyzed salt, or to the astringent character of the soluble beryllium salts. The latter two possibilities appear most probable.

Likewise there is little evidence of correlation between weight gain and the amount of beryllium ingested, since increasing dosage failed to produce a decreasing weight gain with any degree of consistency.

Table 4.—*Results of oral administration of beryllium compounds*

Beryllium compound ingested	Percent of beryllium compound in diet	Estimated daily consumption of beryllium	Time on test	Total beryllium ingested	Initial weight	Net change in weight (grams)		Percent mortality on test	Mg. Be/10 gm. tissue				Mg. beryllium in skeleton
						Test animals	Control animals		Liver	Lung	Kidney	Bone	
Guinea pigs													
	Percent	*Mg.*	*Weeks*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Percent*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
Beryllium carbonate	0.31	10	29	2.03	320	512	501	0	0.005	0.009	0.001	0.052	0.080
Do	0.61	20	29	4.06	360	418	501	0	0	0.008	0.004	0.180	0.180
Do	0.92	30	21	4.41	481	461	521	25	0	0	0.012	0.018	0.026
Beryllium phosphate	0.32	10	14	0.98	696	212	171	0	0.005	0	0	0.024	0.043
Do	0.96	30	13	2.73	743	279	171	0	0.008	0.003	0	0.080	0.118
Do	0.96	30	21	4.41	202	504	521	0	0	0	0.015	0.086	0.206
Beryllium sulfate	0.47	10	17	1.19	711	214	222	0	0.003	0	0.001	0.043	0.067
Do	1.40	30	27	5.67	674	274	420	25	0.059	0.020	0.020	0.140	0.265
Do	1.40	30	14	2.94	172	208	393	25	0.088	0.010	0.061	0.610	0.529
Beryllium oxyfluoride	0.10	1.1–5.5	11	0.24	444	99	217	25	0.019	0	0.003	0.079	0.117
Rats													
Beryllium carbonate	0.77	10	18	1.26	165	61	65	0	0.006	0	0	0.056	0.058
Do	1.53	20	18	2.52	160	90	65	0	0.012	0.004	0.005	0.099	0.125
Do	2.30	30	18	3.78	131	55	65	0	0.050	0.004	0.012	0.593	0.556
Beryllium phosphate	0.80	10	18	1.26	138	108	65	0	0.016	0.012	0.004	0.038	0.036
Do	1.60	20	18	2.52	131	122	65	0	0.005	0	0.006	0.080	0.086
Do	4.01	50	18	6.30	185	2	65	0	0.018	0.028	0.004	0.138	0.122
Beryllium oxide	0.83	30	16	3.36	186	41	53	0	0	0.012	0	0	0
Beryllium chloride	0.44	5	16	0.56	183	32	53	0	0	0	0	0	0
Beryl	7.50	30	16	3.36	179	39	53	0	0.014	0.004	0.004	0.025	0.022
Beryllium hydroxide	1.43	30	16	1.12	179	56	53	0	0	0	0.002	0.002	0.002
Beryllium sulfate	3.51	30	16	2.36	185	23	53	0	0.016	0.004	0.022	0.147	0.196

This is probably related to the fact that most of the beryllium is excreted unabsorbed. (See p. 40.) Notable exceptions occurred with the rats that were fed 50 mg. beryllium phosphate and 30 mg. beryllium sulfate daily. However, owing to an epidemic of rat bite fever which occurred in the rat colony, among control and test animals alike, it was possible that the loss of weight both with the controls and with the beryllium rats was due to severe illness which was in no way connected with beryllium.

The large differences in weight gain for the various groups of animals are explained by their comparative initial weights, since young animals gain more than older animals over a like period of time. It may be concluded that, in general, the ingestion of insoluble beryllium compounds did not inhibit the growth of guinea pigs and white rats. However, in the case of the soluble salts, there was a definite retardation of growth. In the form of insoluble salts, such as the phosphate and carbonate, daily doses of beryllium up to 40 mg. per kg. of body weight (equivalent to a daily intake of 22 gm. of beryllium carbonate by a man weighing 70 kg.) had no effect on the rate of growth.

The mortality for the entire group of test animals was 4 percent and their general health did not appear different from that of the controls. One exception to the above occurred with the guinea pigs which ingested 50 mg. beryllium daily, as the phosphate. After three weeks they became very listless and began losing weight. The reason for this was traced to the fact that the pigs were not eating their food, probably because the large amount of beryllium phosphate made it unpalatable. Upon reduction of the dose to 30 mg. beryllium daily, they resumed eating and began gaining weight in a normal fashion. Guinea pigs ingesting 1.1 mg. of beryllium as beryllium oxyfluoride gained weight normally, but lost weight when the dose was increased to 5.5 mg. daily.

In order to compare the hematology of test and control animals, blood samples were taken before and after the test period. Blood smears, hemoglobin estimations (Newcomer method), and erythrocyte counts were made. The average results of the latter two are shown in table 5. For comparison with these figures, Craige (*22*) gives an erythrocyte count range of 4.5 to 6.8 millions per $mm.^3$ and a hemoglobin range of 15.6 to 17.3 gm. per 100 ml. of blood for normal guinea pigs. His normal range for the erythrocyte count of rats is 7.0 to 10.0 millions and the hemoglobin range from 15.6 to 19.0 gm. In contrast with Craige's figure, von Oettingen et al. (*126*) recently found that counts made on normal rats gave an average value of 7.7 million erythrocytes per $mm.^3$, with 5.3 and 9.5 millions as extremes. The hemoglobin showed an average value of

12.9 gm. per 100 ml. of blood with a range from 11.0 to 16.0 gm. per 100 ml. Although all hemoglobin estimations are lower than the normal range given by Craige, they conform rather closely to those given by von Oettingen. It is apparent that no significant differences exist between test and control animals, either before or after exposure. Erythrocyte counts on rats were greater after exposure than before, but since control animals show the same tendency, this may be attributable to the ration rather than to the ingested beryllium. A study of the morphology of the stained blood smears showed a mild secondary anemia, but again there appeared to be no difference between control and test animals and in general, it would appear that the ingestion of beryllium compounds produced no hematopathological changes.

Table 5.—*Comparison of blood changes in rats and guinea pigs following oral administration of various beryllium compounds*

Experimental conditions	Beryllium fed daily	Number of animals	Hemoglobin (Newcomer)	Erythrocytes
	Rats			
	Mg.	*Number*	*Gm. per 100 ml.*	*Millions per mm.³*
Pre-exposure		12	14.1	7.0
Beryllium carbonate	30	5	13.0	8.0
Beryllium phosphate	50	5	14.9	8.0
Beryllium sulfate	30	2	11.7	6.7
Beryllium oxide	30	2	13.2	8.2
Beryllium hydroxide	10	2	14.7	9.3
Beryllium chloride	5	3	14.2	8.2
Beryl	30	2	12.4	8.2
Control rats		5	13.3	8.9
	Guinea pigs			
Beryllium carbonate	10	3	11.7	5.4
Do	20	4	12.5	5.4
Do	30	3	14.2	5.5
Beryllium phosphate	10	4	12.4	5.7
Do	30	7	12.5	5.4
Beryllium sulfate	10	4	12.6	5.8
Do	30	3	12.8	6.2
Control guinea pigs		9	12.5	5.3

Tissue distribution of beryllium.—A general inspection of the analytical results shown in table 4 reveals three facts of interest:

1. The amount of beryllium retained by the body is very small when compared to the total amount ingested. By dividing the total amount of beryllium found by the total ingested the percent retained is found to be about 0.006. The obvious inference from this is that not much beryllium is absorbed from the gastro-intestinal tract, or that, if absorbed, it is largely excreted in the urine. Analyses of typical urine samples were usually negative, thus the former would appear to be more probable. This was confirmed by later work on the degree of absorption of ingested beryllium by a dog (p. 40).

Apparently beryllium behaves much like magnesium in this respect.

2. In general, the amount of beryllium retained by the body increases with the dose. This tendency is marked in the case of the bone tissue, but results of soft tissue analyses are less conclusive. Although average values show the same trend, individual animals in each group varied widely and practically all groups yielded soft tissues in which there was no beryllium. From this it appears that there is a definite storage of beryllium in the bones, but that beryllium found in the soft tissues is more or less transient.

The comparative ratios of recovered beryllium to the total ingested are given in table 6. If the amount of beryllium retained is proportional to the dose given, these ratios should be constant for each beryllium compound. That this is not the case for the soft tissues is immediately apparent, but with a few notable exceptions, the amount of beryllium found in the bones is approximately a linear function of the amount ingested. (See table 4.)

Table 6.—*Ratio of the beryllium content of 10 gm. portions of tissue to the total ingested beryllium* $\times 10^6$

Compound	Daily dose of Be	Total beryllium ingested	Mg. Be/10 grams tissue / Total mg. Be ingested $\times 10^6$			
			Liver	Lung	Kidney	Bone
	Guinea Pigs					
	Mg.	*Gm.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
Beryllium carbonate	10	2.03	2.50	4.43	0.49	25.60
Do	20	4.06	0	1.97	.99	29.60
Do	30	4.41	0	0	2.71	4.10
Beryllium phosphate	10	.98	5.10	0	0	24.50
Do	30	2.73	2.90	1.10	0	29.30
Do	30	4.41	0	0	3.40	19.50
Beryllium sulfate	10	1.19	2.50	0	.84	36.10
Do	30	5.67	10.40	3.53	3.53	24.70
Do	30	2.94	30.00	3.40	20.70	207.00
	Rats					
Beryllium carbonate	10	1.26	4.76	0	0	44.40
Do	20	2.52	4.76	1.59	1.98	39.30
Do	30	3.78	13.20	1.06	3.18	158.10
Beryllium phosphate	10	1.26	12.70	9.52	3.17	30.20
Do	20	2.52	1.98	0	2.38	31.70
Do	50	6.30	2.86	4.44	.64	21.90
Beryllium oxide	30	3.36	0	3.57	0	0
Beryllium chloride	5	.56	0	0	0	0
Beryl	30	3.36	4.16	1.19	1.19	7.04
Beryllium hydroxide	10	1.12	0	0	1.79	1.79
Beryllium sulfate	30	3.36	4.76	1.19	6.56	43.80

The distribution of beryllium in the various tissues is shown in table 4 and in figure 6. Taking the beryllium content of the lungs as 1, the ratio of beryllium content of lung, kidney, liver, and bone is found to be approximately 1:1.5:3:20 for the entire group of animals. The corresponding ratio for the guinea pigs alone is

1:2.5:4:25 and for the rats it is 1:0.86:2:17. Although some disparity was apparent, the relatively high value for the bones further bears out the fact that storage of absorbed beryllium is largely in the bones rather than in the soft tissues.

3. There appears to be no constant relation between beryllium content of the tissues and the general health of the animals. No correlation was found between weight gain and beryllium recovered later

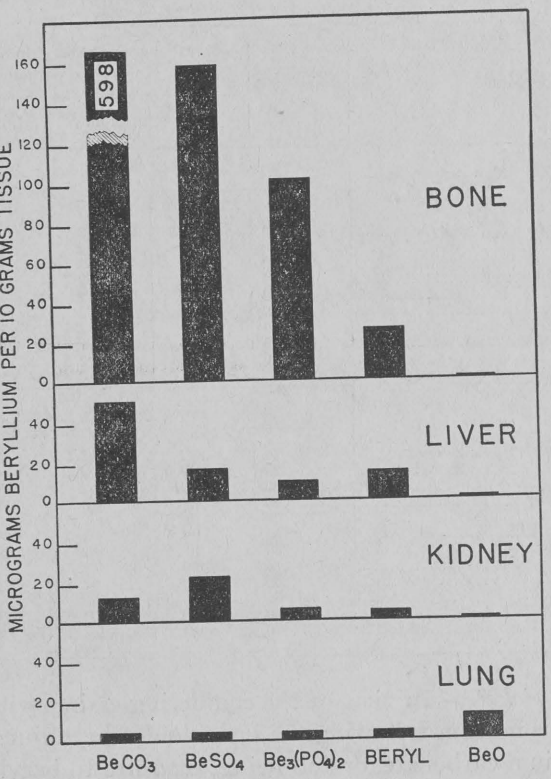

FIGURE 6.

The tissue distribution of beryllium in rats after daily ingestion of various amounts of beryllium compounds (30 milligrams of beryllium) for 17 weeks.

from bones and soft tissues. Pathological examination also failed to reveal such a relationship, since animals showing definite pathology often contained no beryllium, while those containing the largest amount were often free of pathology. This is illustrated in table 7, where the ratios of μgm. beryllium per 10 gm. tissue to "total pathology" are given for six groups of animals. By "total pathology" is meant the sum of all possible retrograde pathological changes as recorded during the usual pathological examination of the individual

tissues. Values in each case are the averages of results for four animals.

In other words, the deposition of beryllium in the tissues is not necessarily accompanied by pathological changes, appears to have little bearing on general health of the animal, and would appear to be more or less incidental in nature.

Table 7.—*Ratio of beryllium content to "total pathology"*

Compound	Amount of Be ingested daily	Micrograms of beryllium/10 grams tissue Total pathology			
		Liver	Kidney	Lungs	Bone
		Guinea pigs			
	Mg.				
Beryllium carbonate	10	5/.5	1/3	5/2.5	80/0
Do	20	0/.5	4/2	0/1	180/0
Do	30	0/1	12/5	0/2.5	25/1.5
Beryllium phosphate	10	5/1	0/2.5	5/3	
Do	30	4/0	8/2	4/2	162/8
Beryllium sulfate	10	3/2	1/2.5	3/2.5	67/0
Do	30	74/1	41/2	74/2	397/1
Beryllium oxyfluoride	1.1–5.5	19/0	3/1.5	19/1.5	117/0
Controls	0	0/0	0/2.5	0/2	0/2
		Rats			
Beryllium carbonate	10	6/2	0/2.5	0/4	56/0
Do	20	12/.5	5/2.5	4/1	99/0
Do	30	50/1	12/2	4/1.5	598/4
Beryllium phosphate	10	16/.5	4/3	12/0	38/0
Do	20	5/.5	6/2.5	0/1	80/0
Do	50	18/.5	4/1.5	28/1	138/0
Beryllium oxide	30	0/2.5	0/2	12/0	0/0
Beryllium chloride	5	0/0	0/2.5	0/0	0/0
Beryl	30	14/.5	4/2.5	4/0	25/1
Beryllium hydroxide	30	0/.5	2/2	0/.5	2/0
Beryllium sulfate	30	16/1	22/1.5	4/2	147/1.5
Controls	0	0/1	0/3	0/1	0/0

Beryllium rickets.—In view of the conflicting claims with reference to a rachitic condition following the ingestion of beryllium salts especially beryllium carbonate, or following exposure to beryllium fumes or dust, it is of interest that the present experimental work has failed to demonstrate beryllium as an etiological factor.

The growth rates (figs. 7 and 8) with beryllium carbonate, beryllium phosphate, and other insoluble beryllium compounds do not indicate any pronounced effect of these ingested salts and closely parallel that of the control animals. No gross evidence of a beryllium effect was noted.

A comprehensive X-ray study was made not only of those animals fed beryllium carbonate but also of animals fed various other beryllium compounds. Animals fed beryllium phosphate, beryllium oxide, beryllium chloride, beryllium hydroxide, beryllium sulfate and beryl as well as the beryllium carbonate animals and the controls were X-

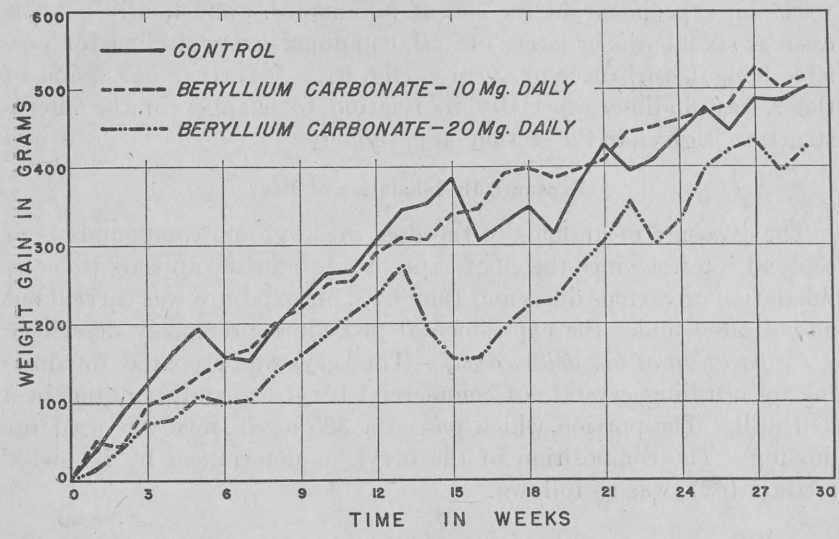

FIGURE 7.

The weight gain, over a 29-week period, of guinea pigs ingesting 10 and 20 milligrams of beryllium daily as beryllium carbonate.

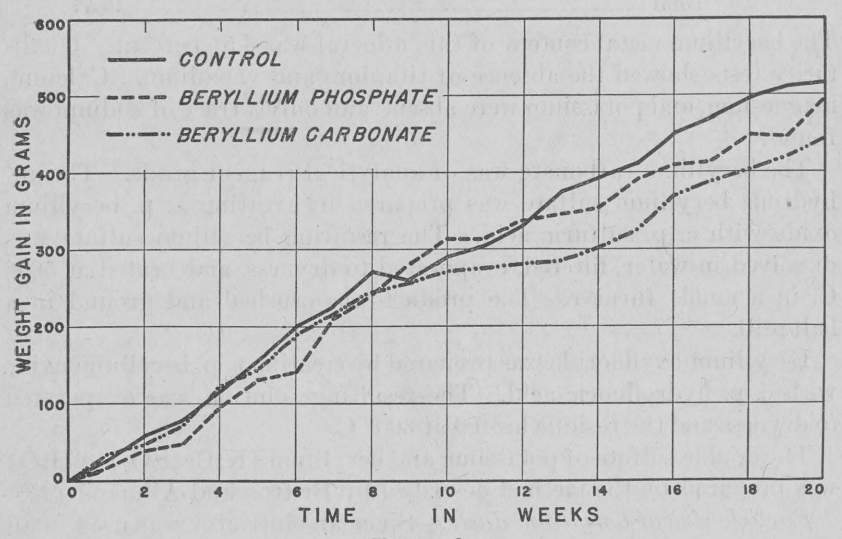

FIGURE 8.

The comparison of weight gains of guinea pigs ingesting 30 milligrams of beryllium daily as carbonate and as phosphate.

rayed before administering the beryllium compound, again after three weeks on experiment, at the end of four months, and finally in some cases at six months or later. In all, 85 animals were studied for possible bone disturbances by X-rays (fig. 9). A further discussion of the X-ray findings especially in relation to changes in the microstructure is given in the section on pathology.

Exposure By Inhalation of Dust

The exposure of animals to the dust of beryllium compounds is of especial interest, since the chief exposure in industry appears to be by inhalation of various dusts and fumes. This exposure was carried out as indicated under the experimental procedure previously described.

Preparation of beryllium dusts.—The beryl was prepared for dusting by crushing crystals of commercial beryl ore and grinding in a ball mill. The portion which passed a 325 mesh sieve was used for dusting. The composition of the beryl, as determined by Knowles' method (*62*), was as follows:

	Percent
BeO	12.51
Al_2O_3	18.19
Fe_2O_3	0.88
SiO_2	64.60
Loss on ignition	1.50
Total	97.77

The beryllium metal content of this mineral was 4.51 percent. Qualitative tests showed the absence of titanium and vanadium. Calcium, magnesium, and potassium were absent, and only a trace of sodium was found.

The beryllium carbonate was of analytical reagent grade. The anhydrous beryllium sulfate was prepared by treating c. p. beryllium oxide with c. p. sulfuric acid. The resulting beryllium sulfate was dissolved in water, filtered, evaporated to dryness, and heated at 500° C. in a muffle furnace. The product was crushed and ground in a ball mill.

Beryllium oxyfluoride was prepared by treating c. p. beryllium oxide with c. p. hydrofluoric acid. The resulting solution was evaporated to dryness and the residue heated at 300° C.

The double sulfate of potassium and beryllium ($K_2Be(SO_4) \cdot 2H_2O$) was prepared by the method described by Britton and Allmand (*12*).

Particle size of beryllium dusts.—Since an elutriator was used in all experiments, the particle size of the various dusts did not vary greatly during the different exposures. Measurements (200 to each slide measured) were made according to the procedure of Bloomfield and Dalla-Valle (*10*). No measurements were made of the sulfate and oxy-

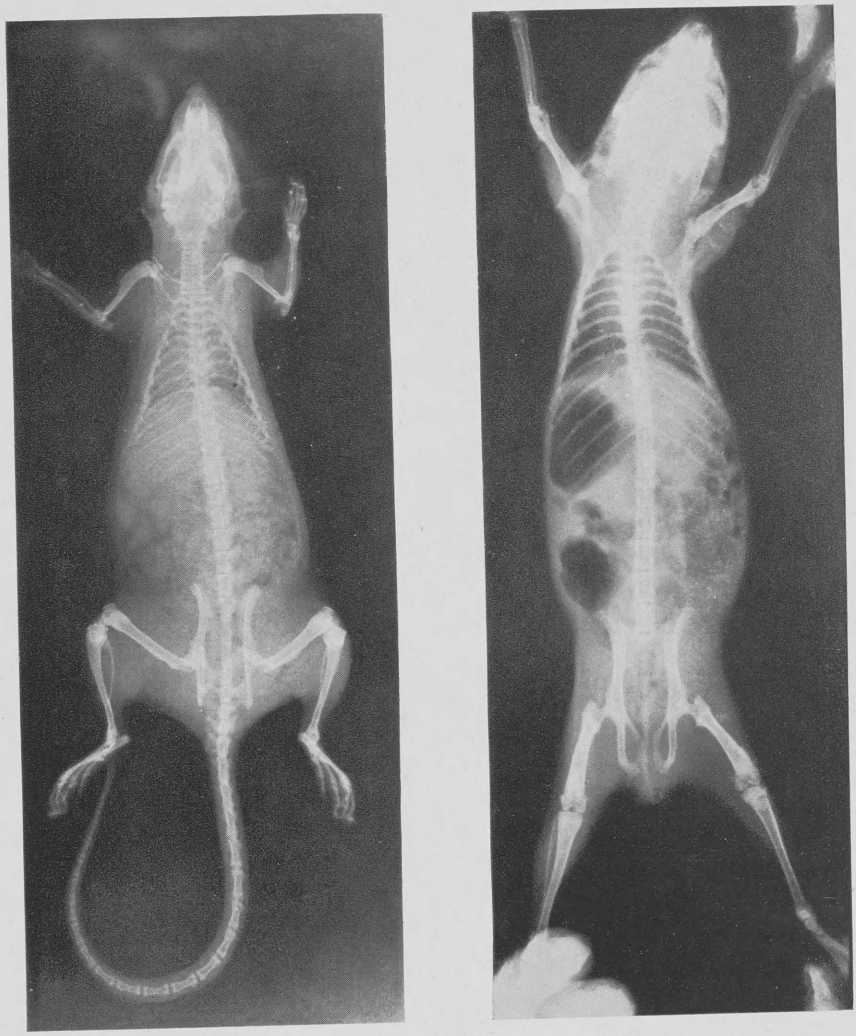

FIGURE 9.

Roentgenograms of rat and guinea pig after having ingested, respectively, 30 and 20 mg. of beryllium as beryllium carbonate daily for 4 months. Pathological examination showed no evidence of rickets.

fluoride dusts, owing to their deliquescent properties but representative samples of the other dusts were measured. These results are shown in table 8.

Table 8.—*Particle size of beryllium dusts*

Type of dust	Average particle size	Deviation	Median
	Microns	*Microns*	*Microns*
Beryllium carbonate	1.53	±0.467	1.47
Beryl	0.99	±0.306	0.92
Electrolysis dust	1.42	±0.612	1.20

It will be noted that the average particle size was somewhat more than one micron. Measurements made at different times indicated that the particle size did not vary significantly from the values given in table 8. However, variations in the physical properties of the different compounds, apart from particle size, made it impossible to subject the animals to exactly the same exposure each time. The actual dose given had to be determined indirectly, as explained below.

Retention of inhaled beryllium dust.—The theoretical amount of beryllium dust retained in the lungs following exposure was compared with the actual lung content as determined by analyses at the conclusion of the experiment. For this purpose, it was assumed that an average guinea pig breathes a total of 2 l. of air per hour (*39*) and that the volume of air moved in ordinary respiration would be somewhat uniform in the various groups since the pigs were initially uniform in size. From this and the known beryllium content of the air, the theoretical amounts of beryllium inhaled were calculated, and are given in table 9. On the basis of these data, in the cases of more than one exposure, the average amount of retention (actual lung content) of the dust was approximately 25.6 percent.

Table 9.—*Retention of various inhaled beryllium dusts*

Compound	Number of exposures	Theoretical beryllium inhaled	Beryllium found in lungs	Retention
		Mg.	*Mg.*	*Percent*
Beryllium carbonate	90	16.70	5.30	31.7
Do	33	9.10	2.64	29.0
Beryl	30	1.59	0.37	23.2
Beryllium oxyfluoride	9	0.16	0.038	24.8
Beryllium sulfate (anhydrous)	1	0.043	0.028	65.1
Potassium beryllium sulfate	6	0.15	0.039	19.3

The apparent discrepancy, in percentage retention, between the anhydrous beryllium sulfate and the other beryllium compounds is doubtless due to the fact that exposures to the other compounds ex-

tended over a long time, whereas in the case of the anhydrous beryllium sulfate, death following the one exposure came too quickly to permit comparable loss from the lungs by absorption or otherwise. The amount of beryllium found in the lungs represents retention in a mechanical sense and does not indicate the amount lost by absorption, by coughing and swallowing, or by retention of the substance in entering the nasal passages or mouth. This calculated retention therefore can represent only a very rough approximation.

Effect on animals.—Guinea pigs were exposed to beryllium dusts for periods of 30 to 40 minutes per day, 6 days a week, over intervals ranging from 1 day to 15 weeks. Significant data concerning the different exposures with reference to mortality are shown in table 10. As will be observed in this table the greatest exposure was made with beryllium carbonate. Two series of exposures were made with this substance. In one, 16 animals were given a total of 90 exposures over a period of 107 days at an average beryllium concentration of 188.9 mg. of beryllium per $m.^3$, with a resulting mortality of 44 percent. In the other experiment 10 animals were given a total of 33 exposures over a period of 39 days at an average beryllium concentration of 233 mg. of beryllium per $m.^3$ In spite of the higher beryllium concentration in the latter experiment there were no deaths among the test animals. The higher mortality in the former experiment was attributed to the duration of the experiment, a greater degree of heat and humidity prevailing at the time of that experiment, and to the development of a dust pneumonia after prolonged exposure. The death rate (table 10) and weight changes (fig. 10) indicate that beryllium carbonate dust exerts a deleterious effect on the animals. With both groups an irritant action was apparent on examination of the lung tissue. However, in neither case did the animals appear to suffer any particular discomfort during the exposure period, except for the slightly labored breathing which might be caused by any dust of like concentration.

On the other hand, a significantly higher mortality rate occurred with exposure to anhydrous beryllium sulfate and to beryllium oxyfluoride as dusts. (See fig. 11.) These exposures were relatively short with a lower concentration of beryllium salt. The first effect was one of irritation to eyes and respiratory systems, followed by increasingly difficult breathing, and finally convulsions and death. As will be noted later these salts were shown to have an irritant action on contact with the human skin.

Both beryllium oxyfluoride and beryllium sulfate give an acid reaction in solution, and it seems that the acute toxicity is due to the effect

Table 10.—*Mortality of guinea pigs following exposure to dusts of various beryllium compounds*

Compound	Number of animals	Number of exposures	Total hours exposed	Average exposure per animal (hours)	Beryllium concentration mg./m.^{3}	Percent mortality		Duration of experiment
						24 hours after 1st exposure	Time of terminating experiment	
			Hours	*Hours*	*Mg. per m.^{3}*	*Percent*	*Percent*	*Days*
Beryllium carbonate	16	90	60	44.2	188.9	0	44	107
Do	10	33	22	19.5	233.0	0	0	39
Beryl	8	30	20	18.8	42.4	0	13	34
Beryllium oxyfluoride	6	9	6	2.9	27.5	33	67	10
Beryllium sulfate (anhydrous)	6	1	.7	.5	43.2	67	67	1
Potassium beryllium sulfate	6	6	3	3.0	24.2	0	0	7
Control	7					0	14	107

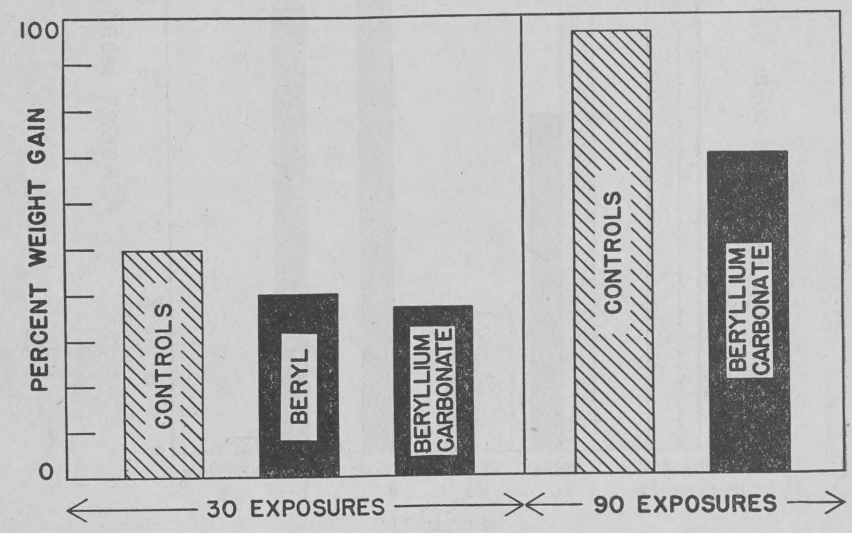

FIGURE 10.

The weight changes of guinea pigs exposed to various beryllium dusts.

of the anion rather than to beryllium, especially since the beryllium concentration was quite low. This assumption was borne out by further exposures of guinea pigs to the double salt, potassium beryllium sulfate ($K_2Be(SO_4)_2 \cdot 2H_2O$). Solutions of this double salt have about the same acidity as the oxyfluoride, while being considerably less acid than beryllium sulfate. The toxic effect of the double sulfate was

less apparent than with the normal sulfate. No deaths occurred in six exposures with the potassium beryllium sulfate, although two-thirds of the animals were killed in a single exposure with the normal sulfate. The toxicity of the fluoride which has a pH similar to that of potassium beryllium sulfate in solutions of the same strength was probably due

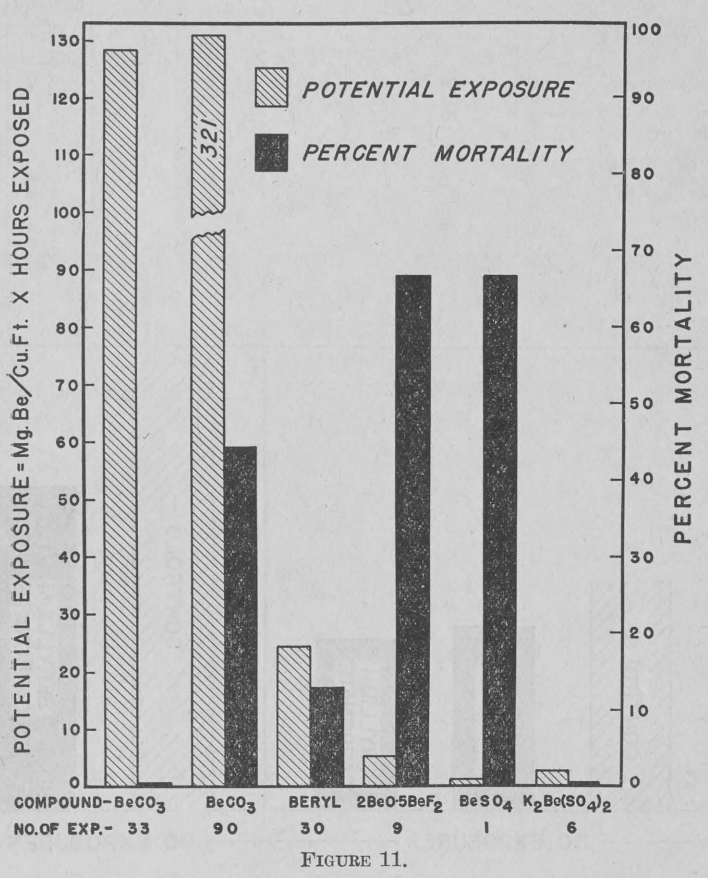

FIGURE 11.

The mortality of guinea pigs following exposure to beryllium dusts.

to the anion since the mortality from this salt was high. Deaths among animals exposed to the carbonate cannot be attributed directly to beryllium poisoning, but rather to pneumonia and other respiratory diseases indirectly caused by the inhalation of dust in addition to the heat and humidity.

Distribution of beryllium in tissue.—The distribution of beryllium in tissue, following the inhalation of beryllium compounds, is shown in

table 11. Results for each group are expressed as mg. per 10 gm. of fresh tissue.

Table 11.—*Distribution of beryllium in the tissues of animals following the inhalation of various dusts*

Compound	Average hours exposed	Mg. Be/10 grams of fresh tissue			
		Lung	Liver	Kidney	Bone
Beryllium carbonate	44.2	7.56	0.045	0.020	0.138
Do	19.5	6.08	0.012	0.007	0.018
Beryl	18.8	0.60	0	0.004	0.017
Beryllium sulfate	0.5	0.068	0	0.002	----------
Beryllium oxyfluoride	2.9	0.07	0.010	0.005	----------
Potassium beryllium sulfate	3.0	0.039	0.012	0.008	0.061

In general, the distribution of toxic substances in the various tissues following ingestion, inhalation, and in some cases intraperitoneal injection, is a valuable means of indicating the nature of the toxic substance as well as the means of defense within the animal organism. In the case of beryllium, the distribution of beryllium in the various tissues was remarkably low even following heavy exposure to various beryllium compounds. Those animals which received the greatest exposure to beryllium, i. e., to beryllium carbonate, had a lung beryllium content at the end of the exposure of 7.56 mg. of beryllium per 10 gm. of fresh tissue in the first case and 6.08 mg. of beryllium per 10 gm. of fresh tissue in the second case. They had stored a relatively small amount in the liver, kidneys, or bones. A somewhat greater amount of beryllium was found in the bones of animals exposed to potassium beryllium sulfate. This may be due to the greater solubility of this salt than that of the other compounds in which analyses were completed. The relatively small amounts stored in these tissues would appear to indicate that either the beryllium salts were so insoluble that they were not absorbed or that, when absorbed, they were rather rapidly excreted. However, no correlation was possible between the absorption and distribution of the soluble and insoluble compounds, owing to the great differences in duration of exposure. The pnemoconiosis characteristic in this test is discussed in the section on pathology.

It may be concluded that, while the insoluble beryllium compounds tested are not more irritant than other dusts, prolonged inhalation of such compounds should be avoided. The soluble compounds, however, are irritant, owing to hydrolysis, and should not be inhaled since they produce a severe reaction within a short time.

VI. TOXICITY OF THE PRODUCTS OF ELECTROLYSIS OF THE MOLTEN FLUORIDES

In the evaluation of the toxicity of beryllium with reference to industrial exposure, it is insufficient to limit this to the ingestion or inhalation of such substances as the carbonate, chloride, or sulfate. The chief type of beryllium extraction process in which poisoning is said to occur in industry (*46*, *47*) is that of electrolysis of mixtures of molten fluorides. It was considered necessary, therefore, to simulate so far as practicable the conditions in which this process is employed. The apparatus for this purpose has been discussed above. Guinea pigs and rats were used as experimental animals and a total of 18 experiments was performed in which the conditions were systematically varied as shown in tables 12 and 13.

Table 12.—*Effect on guinea pigs of fumes and dust from the electrolysis of molten fluorides*

Exposure No.	Crucible charge of fluorides	Type of exposure	Volume air in cu. ft.	Mg. of beryllium, per cu. ft.	Mg. of fluoride, per cu. ft.	Length of exposure	Number of animals	Mortality	Beryllium/10 grams of lung tissue	Fluoride/10 grams of lung tissue
				Mg.	*Mg.*	*Min.*		*Pct.*	*Mg.*	*Mg.*
1	Ba, Be, Na	Fumes and dust	12	0.033	1.5	30	4	100	0.018	0.025
2	Ba, Be, Na	do	13	0.125	2	52	1	100	0.09	0
3	Ba, Be, Na	do	15	--------	3	60	1	100	0.06	--------
4	Ba, Be	do	12	0.48	0.8	50	3	100	0	0.07
5	Ba, Na	do	12	0	0.53	65	2	100	0	0.15
6	Be, Na	do	22.5	0.2	3	90	2	100	0.12	0.05
7	Ba, Be, Na	Filtered fumes	15	0	0.7	60	3	0	--------	--------
8	Ba, Be, Na	Anode fumes	12	0	2	60	2	0	--------	--------
9	Ba, Be, Na	do	18	0	5	90	2	0	--------	--------
10	Ba, Be, Na	do	30	0	0.07	150	2	0	--------	--------
11	Ba, Be, Na	Cathode fumes	15	0.1	3	60	2	0	--------	--------

Table 13.—*Exposure and tissue distribution data with reference to the effect on rats of fumes and dust from the electrolysis of molten fluorides*

Exposure number	Electrolysis data					Rat number	Tissue distribution			
	Crucible charge of fluorides	Length of exposures	Volume air in cu. ft.	Mg. of beryllium per cu. ft.	Mg. of fluoride per cu. ft.		Mg. Be/10 grams tissue			Fluoride/10 grams of lung tissue
							Liver	Kidney	Lung	
		Minutes					*Mg.*	*Mg.*	*Mg.*	*Mg.*
1	Ba, Be, Na	52	13	0.125	2.6	1	0	0	0.16	0.2
2	Ba, Be, Na	20	5	0.009	1.2	2	0	0.035	0.34	--------
3	Ba, Be, Na	60	10	0.73	1.33	3	0	0	0.11	0.1
4	Ba, Be, Na	60	10	0.65	2.2	4	0	0	0.22	0.6
5	Ba, Be, Na	60	15	0.20	3.65	5	0	0	0.26	0.2
6	Ba, Be, Na	60	15	--------	3.0					
7	Ba, Be, Na	60	15	0.102	2.7	Average	--------	--------	0.22	0.28
		Hours								
Total time	--------	6⅓	--------	--------	--------					

Sodium, beryllium, and barium fluorides were used together in one series of exposures, and beryllium and barium fluorides, sodium and barium fluorides, sodium and beryllium fluorides in succeeding experiments. These variations in procedure, in which each of the cations was successively eliminated in the melt, were undertaken in order to determine whether the toxic effect of the fume was primarily due to the metal or to the fluoride. The effect of removing dust from the fume products of electrolysis (by passing the dusty air through cotton wool before it entered the exposure chamber) is indicated in table 12. It is clear that the dust rather than the gas is a major factor in the irritant action of the products of electrolysis.

Examination of the gases and fumes evolved during electrolysis.—The air in the chamber was analyzed for carbon monoxide with a Mine Safety Appliance Carbon Monoxide Indicator. The highest carbon monoxide content noted was 0.01 percent which is within the safe limit given by Haldane (*53*) and by American Engineering and Industrial Standards (*3*). The air entering the exposure chamber was free from oxides of nitrogen as was shown by experiments in which measured volumes of air were drawn through dilute potassium hydroxide solution and oxidized with hydrogen peroxide. When tested by the phenol disulfonic acid method, the results obtained were negative indicating the absence of oxides of nitrogen. Analysis of the water in the absorption train for its fluoride content, made by the zirconium alizarin lake method of Elvove (*32*), and the analyses for beryllium of aliquot portions by the fluorescence method, indicated that the fluoride content varied throughout the experiments between 0.07 and 5.0 mg. and the beryllium content varied between 0.03 and 0.73 mg. per cu. ft. The fumes given off during electrolysis were shown to consist of a true vapor phase and a very fine smoke or dust.

The dust collected in the chamber was analyzed by the method of X-ray diffraction[4] and was shown to contain beryllium fluoride (BeF_2), beryllium oxide (BeO), barium fluoride (BaF_2), and sodium fluoride (NaF). It is to be noted that neither metallic beryllium nor beryllium oxyfluoride was found in the dust. Investigators (*46*, *47*, *133*) have claimed both to be present and the presence of either one or both under different conditions is not to be discounted. Furthermore, the possibility of formation of carbon tetrafluoride or of carbonyl fluoride during electrolysis of the molten fluorides was not overlooked.

Effect on animals.—With a single exception, all guinea pigs were killed within 90 minutes or less, when exposed to the dust and fumes. In every case, the first reaction of the animals was one of irritation to

[4] We are indebted to Junior Physicist W. C. White for his kindness in examining this material.

the eyes and nasal passages. Eyes watered and the animals pawed at their noses. After a few minutes, breathing became labored and the animals coughed occasionally. Breathing gradually became more difficult and the animals became languid. The final stage was usually one of convulsions after which the animals died within a few minutes. Analyses of the lungs of those animals which had died or were sacrificed following exposure to the dust and fumes arising from the electrolysis of molten fluorides showed that the lung content varied between 0 and 0.34 mg. of beryllium per 10 gm. fresh tissue and had a fluoride content of from 0 to 0.6 mg.[5]

When only anode fumes were passed into the chamber, the first symptoms were the same as above, i. e., eyes watered and the animals pawed at their noses. Breathing became labored but in no case did the condition of the animals become acute during the exposures and all appeared to return to normal almost immediately upon removal from the chamber. Comparison of exposure of animals to the separate anode and cathode fumes showed that the anode fumes were especially irritating and would probably cause death at higher concentrations. However, the experimental limitations of this apparatus made it too difficult to attain a sufficiently high concentration of anode fumes for direct comparison with the mixed dust and fumes.

While rats were definitely affected by the fumes, they were more resistant than guinea pigs. Their breathing was labored, they became weak and languid but managed to survive exposure to concentrations of dust and fumes that were fatal to guinea pigs. In brief, the fumes from the electrolysis of beryllium oxyfluoride in a mixture with sodium fluoride and barium fluoride are lethal to guinea pigs in a relatively short time, while rats resisted the action of the fumes better than guinea pigs but on prolonged exposure showed the same general reaction.

The toxic effect was apparently due to the fluoride dust and fumes, for the elimination of any one metal from the melt did not lessen the toxicity appreciably. This was greatly reduced when all dust was removed from the fumes. The toxic action was not due to beryllium metal fume present in the fumes from the electrolysis of beryllium oxyfluoride, since, as has been stated, study of the X-ray diffraction patterns of this dust showed it to be beryllium-free. Furthermore, it would appear that beryllium plays but little if any part in the toxicity of the electrolysis fumes, since elimination of it from the melt did not appreciably lessen the toxic effect.

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[5] These analyses of fresh lung tissue were most conveniently made by macerating the tissue with 5 percent ammonium acetate solution, drying and ashing in an electric muffle furnace at 600° C. The hydrochloric acid solution of this ash was analyzed for its fluoride content by the method of Elvove (*32*).

VII. PHYSIOLOGY

Irritant Action of Beryllium Salts on the Skin

According to Gelman, Pack (*95*) found papulovesicular forms of dermatitis on the face and neck as well as on the back of the hands and on the wrists of workers engaged in the preliminary phases of beryllium extraction. Ulcers resembling those caused by chromates and dichromates occurred on the fingers of these workers. Cutaneous and ocular affections were rarely found in the final phases of beryllium extraction.

In view of the pronounced irritant action of the dust and fumes referred to on pages 32 and 38, a number of patch tests of the more irritant beryllium salts was made on various individuals. Beryllium oxyfluoride and beryllium sulfate were used and compared with beryllium oxide. Both these salts hydrolyze in water, since the solutions are markedly acidic. Since initial patch tests on one individual had shown marked irritation in the case of beryllium oxyfluoride in 18 hours, patch tests of 1, 2, 4, and 6 hours duration in the case of the oxyfluoride, and 6 and 24 hours duration with the sulfate and oxide were made on six individuals. These results are summarized in table 14. In all cases the reactions of the beryllium compounds were observed on the day following the removal of the patch and also later, to note delayed action. Four individuals developed a positive reaction in the case of beryllium sulfate after 24 hours contact while three were positive after the 6-hour exposure. All were negative to beryllium oxide. Beryllium oxyfluoride was revealed as a primary irritant to the skin in all cases with contact for 4 hours or longer, while six cases of skin irritation were apparent in shorter periods of time. Both the sulfate and the oxyfluoride produced redness characteristic of the irritant action of the corresponding free mineral acids.

Table 14.—*Irritant action of beryllium salts on the skin*

Compound	Time of contact in hours	Individuals tested						Number tested	Positive reaction
		A	B	C	D	E	F		
									Percent
Beryllium oxyfluoride	18	+						1	100
Do	6	+	+	+	+	+	+	6	100
Do	4	+	+	+	+	+	+	6	100
Do	2	+	+	+	+	−	−	6	67
Do	1	−	+	−	+	−	−	6	33
Do	24	+	+	+	+	−	−	6	67
Beryllium sulfate	6	−	+	+	+	−	−	6	50
Do	24	−	−	−	−	−	−	6	0
Beryllium oxide	6	−	−	−	−	−	−	6	0
Do									

It would thus appear that, in industry where there is exposure to beryllium oxyfluoride dust or fume, or to beryllium sulfate dust, suitable measures should be employed to protect workers against its irritant action.

Absorption of Beryllium From the Gastro-Intestinal Tract

In order to study the absorption of beryllium from the gastro-intestinal tract, a solution of beryllium sulfate tetrahydrate ($BeSO_4 \cdot 4H_2O$) was administered to guinea pigs by means of a stomach tube. The solution contained 10 mg. of beryllium per ml., and 0.5 ml. was given to each guinea pig. The solution was injected from a syringe through a 2.5 inch 18-gauge hypodermic needle into the esophagus. A drop of solder on the end of the needle prevented injury to the esophagus.

At various intervals, the guinea pigs were sacrificed, the gastro-intestinal tract dissected out, and the mesentery removed. The entire tract was ashed, dissolved in N HCl, diluted to 1 l. and analyzed by the fluorescence method. It was found to be advantageous to starve the animals for 48 hours before administering the dose, this method giving an optimum condition for absorption and simplifying the analyses.

Absorption was determined by subtracting the amount of beryllium recovered from the amount administered. These values are corrected for the recovery of beryllium from the gastro-intestinal tract of an animal sacrificed immediately after the dose was given. Table 15 gives the results for various time intervals. In general these results show no constant value for the rate of absorption either on a milligram per hour, or on a milligram per kg. body weight per hour basis. Although the total amount of beryllium absorbed was small, yet some trend was apparent. Since the numerical values vary widely, the rate of absorption of beryllium probably varies from animal to animal.

Table 15.—*Degree of absorption following the administration of beryllium sulfate into the stomachs of guinea pigs*

Guinea pig number	Weight of animals	Time after administration	Beryllium given	Beryllium found	Beryllium absorbed
	Gm.	*Hours*	*Mg.*	*Mg.*	*Percent*
1	400	0	5	4.5	0
2	455	1	5	3.5	20
3	575	1.5	5	3.5	20
4	455	2	5	4.5	0
5	470	2	5	3.0	30
6	470	3	5	3.5	20
7	530	24	5	1.75	55
8	420	48	5	2.0	50

Excretion of Beryllium

In order to ascertain the mode and rate of excretion of beryllium fed as a soluble salt, a 6 kg. dog was fed 30 mg. of beryllium as beryllium sulfate tetrahydrate (0.6 gm. $BeSO_4 \cdot 4H_2O$) by means of a

gelatin capsule. Specimens of urine and feces were collected at convenient intervals and their beryllium content determined. Results are given in table 16.

Table 16.—*The excretion of beryllium by a dog following the oral administration of 30 mg. of beryllium as beryllium sulfate*

Time interval	Urine volume	Beryllium found in urine	Feces dry weight	Beryllium found in feces	Beryllium excreted
Hours:	*Ml.*	*Mg.*	*Gm.*	*Mg.*	*Percent*
0 to 24	280	0	31.0	15.5	51.7
24 to 30	122	0.03	19.4	11.7	39.1
30 to 48	[1] 230	0.23	10.5	1.0	4.1
48 to 96	525	0.13	50.7	0	0.43
96 to 99	95	0.03	0	0	0.1
99 to 120	215	0.05	48.5	0	0.17
Total	1,267	0.47	160.1	28.2	95.60

[1] Contaminated with feces.

It is apparent that over an interval of 5 days 96 percent of the entire amount of ingested beryllium was excreted. The beryllium was excreted mainly in the feces (94 percent) and very little was excreted in the urine (1.6 percent). Lorenz (*72*) also found that the amount of beryllium in the urine of cats was very small compared with the amount in feces although his determinations were not quantitative.

The general effect of the beryllium sulfate on the dog was of interest in that its similarity to magnesium sulfate was demonstrated. The first stools, collected within 6 hours, were formed, but stools excreted during the next 48 hours were unformed. Except for a slight listlessness observed during the first 6 hours, and the laxative action mentioned above, the dog appeared to suffer no ill effects from the ingestion of beryllium sulfate.

Absorption of Beryllium Carbonate From the Lungs

In an effort to determine the rate of absorption of beryllium from the lungs of animals exposed to beryllium carbonate dust, four guinea pigs were given a 1-hour exposure to dust in which the beryllium concentration was 3.15 mg. per cu. ft. The pigs were sacrificed at the following intervals: immediately, 1, 3, and 24 hours after exposure, and the soft tissues were analyzed for beryllium.

The values obtained from the analyses of these tissues are given in table 17. Apart from the beryllium content of the lungs the soft tissues were free from beryllium in most instances. It is therefore evident that absorption of beryllium carbonate from the lungs is not appreciable within a 24-hour period. The beryllium carbonate thus appears to have no greater solubility in the lung tissue fluid than it has in pure water and in this sense behaves as an inert substance.

Table 17.—*Degree of absorption of beryllium from the lungs of guinea pigs exposed to beryllium carbonate dust*

Pig number	Time sacrificed after exposure	Mg. Be/10 grams of tissue			
		Lung	Liver	Kidney	Blood
		Mg.	*Mg.*	*Mg.*	*Mg.*
1	Immediately	0.05	0	0	0.004
2	1 hour	0.07	0	0	0
3	3 hours	0.10	0	0	0
4	24 hours	0.16	0	0.03	0

Solubility of Beryllium Compounds in Blood Serum and Gastric Juice

Since solubility is an important factor influencing the absorption of any compound, an attempt was made to determine the solubility of beryllium compounds in calf serum and in artificial gastric juice (*54*).

Three-gram samples of beryllium carbonate, beryllium oxide, and beryl were added to 200-ml. portions of serum and gastric juice, respectively, and like amounts of beryllium sulfate and phosphate were added to serum. After the addition of preservatives the bottles were sealed, placed in a constant temperature bath at 25° C., and agitated for 14 days by a mechanical shaker, at which time it was assumed that equilibrium had been reached. The solutions were centrifuged, filtered through cellophane in a high pressure Zsigmondy membrane ultra filtration apparatus, and the filtrates analyzed for beryllium by the fluorescence method. With one exception, beryllium was not found in the filtrates. Beryllium was present in the beryllium sulfate filtrate but it was found that some of the dissolved beryllium sulfate merely precipitated the protein present in the serum. (See page 43.) Therefore no true solubility value could be obtained. Since the other filtrates were free from beryllium, it must be assumed that the beryllium compounds remained undissolved, or that the dissolved beryllium reacted with the protein present to form a precipitate. Experiments described elsewhere indicate that the latter is a strong probability. In any event, the fact that no beryllium passed through the cellophane membrane indicates that any beryllium originally dissolved no longer remained in true solution at the time of filtration. Similarly it would appear that whatever beryllium compound dissolved in the hydrochloric acid of the gastric juice was precipitated or no longer held in true solution. Since the soluble beryllium salts react with proteins and since the less soluble salts show no appreciable solution in serum or gastric juice, it is not surprising that the absorption of beryllium in passing through the alimentary tract is so slight.

Action of Beryllium Upon Blood Serum Proteins

Several metallic poisons are rendered insoluble owing to their reaction with proteins in the alimentary tract, the intestinal walls, or the

internal organs. In certain cases the degree of toxicity is related to the tendency of the metallic salt to form irreversible precipitates with proteins; in other cases marked effects may be produced in extreme dilutions on proteins or more particularly isolated cells. Since, however, the toxicity of different metals depends largely upon their absorbability, the precipitability of various beryllium salts with proteins is not without interest.

Richter (*99*) reported that beryllium nitrate in physiological saline solution when added to ox serum produced a precipitate immediately when the concentration of beryllium nitrate $Be(NO_3)_2$ was varied from 1:20 to 1:200. A light turbidity was produced after 5 minutes at 1:500, while there was no effect at concentrations of 1:1000 to 1:1,000,000. Volter (*125*) has also shown that beryllium chloride and fluoride precipitate serum proteins, and states that beryllium salts denature cell protoplasm.

Similar experiments were made in this investigation using rabbit serum, and at the same time the action of zinc and magnesium nitrates was compared with that of beryllium nitrate.

Stock solutions (1:20) of beryllium nitrate and zinc nitrate were prepared by dissolving 7 gm. $Be(NO_3)_2 \cdot H_2O$ and 7.85 gm. $Zn(NO_3)_2 \cdot 6H_2O$, respectively, in 100 ml. of physiological saline solution. Each of these salt solutions contained 5 gm. of the anhydrous salt in 100 ml. of the saline solution. Suitable dilutions of this stock solution were made. A saturated solution of magnesium nitrate in physiological saline solution was also prepared.

The tests were conducted by adding 1 ml. of serum to 10 ml. of the different salt solutions, and observations were made immediately and at the end of 1 hour. These results are summarized in table 18.

Table 18.—*Comparison of the action of beryllium, zinc, and magnesium nitrates upon rabbit blood serum*

Concentration = gm. of salt: ml. physiological saline solution	Effect of adding 1 ml. of serum to 10 ml. of solutions of—					
	Beryllium nitrate		Zinc nitrate		Magnesium nitrate	
	Immediate	1 hour	Immediate	1 hour	Immediate	1 hour
Saturated	++++	++++	++++	++++	−	−
1 : 20	++++	++++	++++	++++	−	−
1 : 40	++	++++	++++	++++	−	−
1 : 50	±	+	++++	++++	−	−
1 : 200	−	±	++++	++++	−	−
1 : 800	−	−	++++	++++		
1 : 2,000	−	−	+++	++++		
1 : 3,000	−	−	++	+++		
1 : 4,000	−	−	+	++		
1 : 5,000	−	−	±	+		
1 : 8,000	−	−	−	−		

NOTE: ++++=precipitate; +++=light precipitate; ++=turbid; +=light turbidity; ±=very slight turbidity; −=no reaction.

The results given in the above table show no immediate precipitate with beryllium nitrate at concentrations less than 1:40. The order of the salts studied as protein precipitants is: magnesium nitrate, least; beryllium nitrate, greater; and zinc nitrate, greatest. The greatest dilution at which precipitation with beryllium nitrate appears is at 1:200 and even in this case the precipitation is not apparent until after 1 hour. It is of interest that zinc nitrate gave an immediate precipitation with rabbit serum even at a dilution of 1:5000, while magnesium nitrate produced no precipitation at any of the dilutions. It may be concluded that while precipitation occurs with beryllium salt solutions and serum proteins, it is not a particularly sensitive reaction.

Comparative Toxicity of Beryllium, Magnesium, and Zinc on Intraperitoneal Injection

The relative toxicities of beryllium, magnesium, and zinc were compared by ascertaining for each of these the minimum lethal dose on intraperitoneal injection of their sulfates, which killed 75 percent

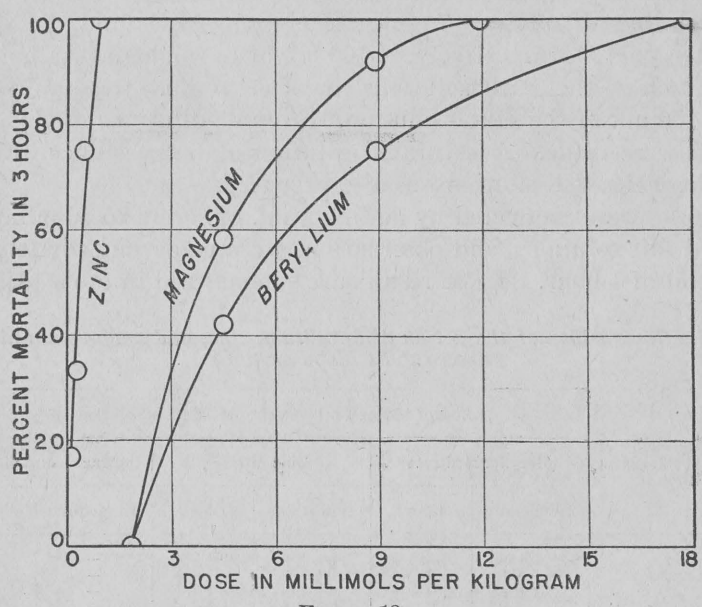

FIGURE 12.

The comparative toxicities of zinc, magnesium, and beryllium sulfate.

of the animals within 3 hours. In order to evaluate these salts properly, equimolar solutions of the three sulfates were used. Molar solutions of beryllium sulfate ($BeSO_4 \cdot 4H_2O$) (buffered with sodium citrate), magnesium sulfate ($MgSO_4 \cdot 7H_2O$) and zinc sulfate

($ZnSO_4 \cdot 7H_2O$) were prepared and from these, suitable dilutions of similar hydrogen ion concentration were prepared and injected intraperitoneally into mice. The animals were observed over a time interval of 3 hours from the time of injection. The order of toxicity diminished from zinc, which was most toxic to beryllium which was least toxic. Graphical representation of the results obtained is given in figure 12. The data derived from these experiments afforded a means of estimating a minimum lethal dose based upon the mortality after 3 hours of a given amount of salt per kg. of body weight. The values thus derived were 8.9 millimols per kg. for beryllium sulfate, 6.1 millimols per kg. magnesium sulfate, and 0.5 millimols per kg. for zinc sulfate.

An experiment similar in many respects to the preceding was made with guinea pigs in which the lethal dose was determined by a survival time of 24 hours. In this experiment, potassium sulfate, beryllium sulfate, magnesium sulfate, and zinc sulfate were injected in known concentrations intraperitoneally and the mortalities determined at the time indicated. It was clear from this experiment that while no irregularity was observable between beryllium, magnesium, and potassium sulfates, the effect of zinc sulfate was pronounced at similar concentrations.

VIII. PATHOLOGICAL CHANGES RESULTING FROM EXPOSURE OF ANIMALS TO BERYLLIUM COMPOUNDS

Tissue from the animals which died or were sacrificed following exposure to various beryllium compounds was submitted for pathological examination. Paraffin sections were made from liver, spleen, lung, kidneys, heart, pancreas, stomach, duodenum, jejunum, ileum, large intestine, portions of the abdominal wall, femur, and sternum. These sections were routinely stained by Lillie's (*69*) eosin-polychrome methylene blue method. Sections from the spleens and livers were stained by ferrocyanide to demonstrate the presence or absence of iron-bearing pigment. A total of 1,672 slides from 223 animals were examined.

Lungs.—A slightly higher incidence of pneumonitis was observed in the lungs of the guinea pigs exposed to potassium beryllium sulfate (fig. 13), beryl, beryllium oxyfluoride, and beryllium carbonate by inhalation than was noted in the controls. There appeared to be no great variation in the number of animals showing pulmonary changes with respect to the different beryllium compounds, the total length of time of exposures (hours), number of exposures, duration of experiment (days), or concentration of beryllium in the atmosphere. The changes observed varied considerably in degree and were characterized by focal or diffuse collections of lymphocytes, varying numbers of

polymorphonuclear leucocytes, amounts of serum, macrophages, and fibroblasts. Congestion of the alveolar capillaries, sometimes with extravasation of blood and serum into the alveoli, was infrequently present in significant amounts. The death rate in these animals was greater than the controls. Appreciable numbers of dust particles ranging from 0.99μ to 1.53μ in size, were noted in the lungs of most of the surviving animals studied. The dust particles were found in the macrophages in the alveolar walls, and in the connective tissue about the small bronchi and blood vessels. Such particles were absent in the lungs of the animals not exposed to dust by inhalation.

No changes were noted in the lungs of the animals exposed to beryllium sulfate although four of the six guinea pigs used in this experiment died within 24 hours following exposure. The lack of pulmonary findings seems to indicate that the hydrolysis products of the compound are so toxic that death ensued before pulmonary damage is produced.

It appears from the pathology observed that the inhalation of potassium beryllium sulfate, beryl, beryllium oxyfluoride, and beryllium carbonate dust increases the susceptibility of guinea pigs to pneumonitis, varying in degree from acute diffuse interstitial pneumonitis to subacute bronchopneumonia.

Guinea pigs exposed to fumes produced by the electrolysis of beryllium, sodium and barium fluorides as used in industry, produced no significant changes in the lungs. Further exposures in which one or the other of the compounds was omitted from the melt likewise showed nothing of note. The mortality was the same regardless of the exclusion of any one of the three compounds. Death occurred during or following a single exposure of from 30 to 90 minutes duration.

A group of rats similarly exposed to the fumes from the electrolysis of the combined beryllium, sodium, and barium fluorides were killed and examined after eight exposures (6⅕ hours). Slight evidence of pulmonary irritation was noted. This is consistent with the longer time to which the rats were subjected to the fumes. The rat appears, therefore, to be more resistant to the fumes produced by the electrolysis of beryllium than the guinea pig.

No appreciable pulmonary changes were observed in guinea pigs fed beryllium sulfate, beryllium oxyfluoride, beryllium carbonate, and beryllium phosphate regardless of the compound, dose or length of time on test. The same was true of the rats fed beryllium chloride, beryllium sulfate, beryllium phosphate, beryllium hydroxide, beryllium oxide, beryllium carbonate, and beryl.

The lungs from groups of guinea pigs receiving a single intraperitoneal injection of beryllium oxide, beryllium carbonate, beryllium phosphate, beryllium hydroxide, beryllium chloride, beryllium

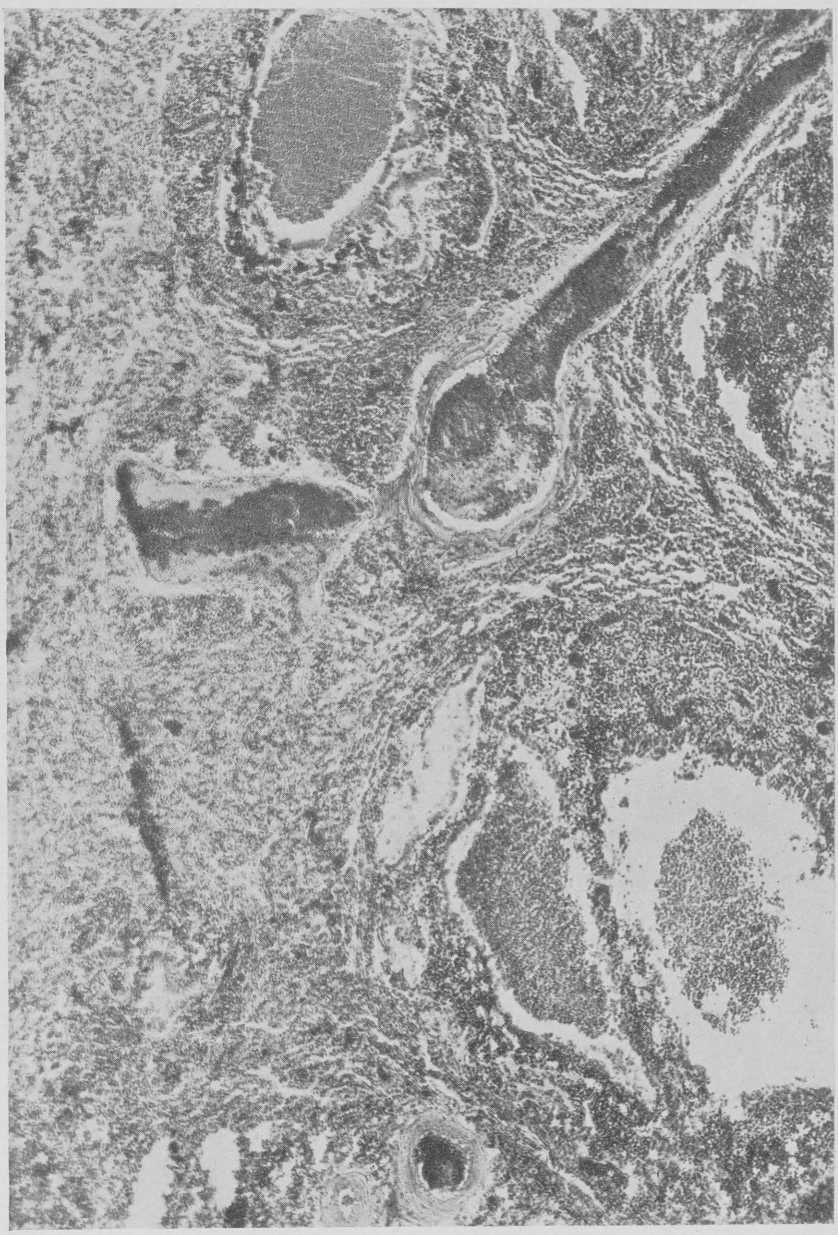

FIGURE 13.

Section of lung following exposure by inhalation of potassium beryllium sulfate.

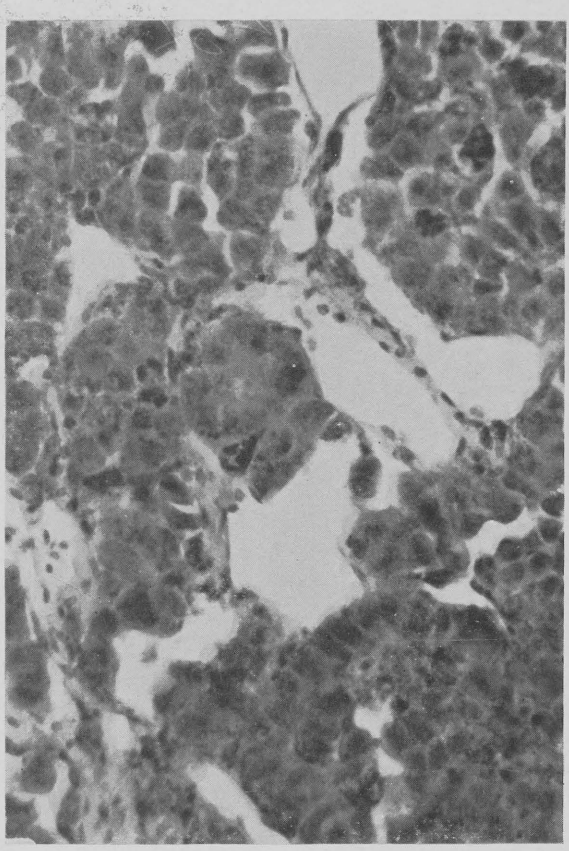

FIGURE 14.

Brown pigment particles present in the zona reticularis of the adrenal cortex.

sulfate, beryllium oxyfluoride, and beryl showed no great variation from the control animals. The incidence of pneumonitis was approximately the same for all compounds including the controls examined over periods of 3 to 32 weeks following injection.

Aside from the presence of dust particles in the lungs of the animals in the inhalation series there was no significant difference in the incidence of pneumonitis whether the beryllium compound was administered by inhalation, ingestion, or intraperitoneal injection.

Kidneys.—A subacute interstitial nephritis characterized by wedge-shaped areas of cellular infiltration, occasionally accompanied by fibroblasts and fibrous connective tissue cells with casts in the straight collecting tubules, was noted in the renal cortex of a number of the animals, but as its incidence approximated that of the controls it could not be attributed to the effects of the beryllium compounds. Route of administration, dose, duration of experiment or species of animal appeared to have no bearing on the occurrence of this finding. Under the conditions of these experiments it can be said that beryllium does not produce nephritis.

Adrenals.—A conspicuous amount of intracellular brown pigment was found in the reticular zone in two of six guinea pigs exposed to potassium beryllium sulfate (fig. 14). The significance of this is questionable as Sharpey-Schafer (*110*) and Maximow and Bloom (*81*) believed this to be normal, although Strong and Elwyn (*117*) considered it a degenerative change. Its presence suggested no correlation with other pathological findings.

Gastro-intestinal tract.—Hemorrhagic necrosis of the gastric mucosa was found in the animals receiving 30-mg. doses of beryllium as the sulfate and 5½ mg. of beryllium as the oxyfluoride by ingestion. This local irritation may be attributed to the hydrolysis products of these compounds. No pathological changes were observed in the gastro-intestinal tract of the other animals studied.

Peritoneum.—Dust nodules were found in the peritoneal cavity of guinea pigs following intraperitoneal injection of the insoluble compounds—beryllium oxide, phosphate, and beryl. These nodules were similar to those produced by dusts causing an inert reaction according to Miller and Sayers (*86*). Nodules of similar size and character were found at autopsy 6 months after injection.

The liver, spleen, and pancreas showed nothing of note.

Bones.—Osteological changes consisting of a thickening of the epiphyseal line by new bone tissue formation with extension of thin, closely packed trabeculae into the shaft and scattered areas of osteogenic tissue were found in 2 of the guinea pigs fed beryllium phosphate and 1 of the rats fed beryllium sulfate (fig. 15). Inasmuch as definite rachitic changes were observed in only 3 animals, the incidence

is too small to be of significance. Roentgenograms were made of 60 rats while on a diet including beryllium carbonate, phosphate, oxide, chloride, hydroxide, sulfate, or beryl, at the beginning of the experiment, at the end of 3 weeks, and at the end of 4 months. Similar examinations were made on 25 guinea pigs fed beryllium phosphate, beryllium sulfate, or beryllium carbonate. In this series films were taken at the beginning of the test and at the end of 3 weeks', 4 months', and 6 months' periods. The daily dose of the phosphate, carbonate, and sulfate varied from 10 to 30 mg. calculated as beryllium. Four of the sixty rats and seven of the twenty-five guinea pigs showed some X-ray evidence of bone disturbance suggesting the possibility of rickets. A few only of these roentgenological findings were confirmed by histological study.

Summary of Pathological Changes

The absence of any pathological change which could be attributed to the action of the beryllium is conspicuous. Even though rickets was observed in a few animals both histologically and by roentgenogram there is insufficient evidence to indict beryllium as the etiological agent.

The effect of prolonged inhalation of excessive amounts of beryllium compounds, as with any other dust, should not be minimized. It should be reiterated that some of the soluble beryllium compounds, because of their hydrolysis products, exert a local irritative action on the body tissues regardless of the mode of administration.

IX. SUMMARY AND CONCLUSIONS

The foregoing investigation indicates that beryllium is of itself not toxic. Animal experiments were made in which a number of beryllium compounds were injected intraperitoneally into guinea pigs at various concentrations; beryllium compounds were administered orally to both young rats and guinea pigs and finally guinea pigs were exposed to the dust of beryllium compounds in high concentrations and over long intervals of time with no indication that beryllium is inherently toxic. Exposure of both guinea pigs and rats to the fumes arising from the electrolysis of molten fluorides containing beryllium fluoride or oxyfluoride show that these fumes are decidedly toxic.

In order to further the study of the effect of beryllium on animal life, it was necessary to develop a convenient method of analysis for minute amounts of beryllium in animal tissues. Two such methods were developed, one a colorimetric, and the other a fluorescence method, each of which is suitable for a certain type of tissue analysis.

The distribution of beryllium in the various organs and tissues of the experimental animals was determined and the values obtained

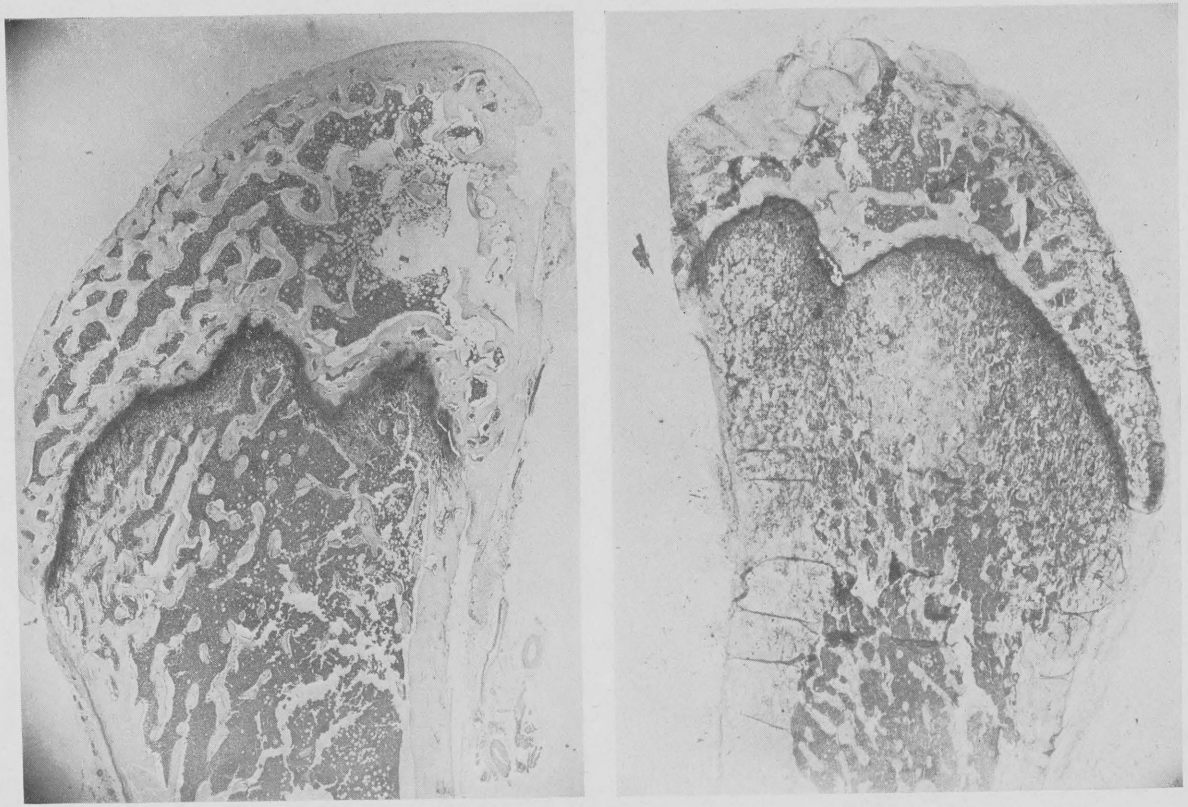

FIGURE 15.

Photomicrographs of bone sections showing normal and rachitic bone.

indicate but very little storage of beryllium following exposure to large amounts whether by mouth, by inhalation, or by intraperitoneal injection. More beryllium was found to be stored in the liver than in the kidneys, and still more beryllium was found in the bone tissue.

No significant change in hemoglobin values was obtained, nor any evidence of polycythemia, or other dyscrasia following exposure to beryllium.

Certain salts of beryllium which hydrolyze easily, such as the fluoride and sulfate, were found to have an irritant effect on the skin but this effect was absent in the case of the neutral salts.

Recovery experiments indicated that absorption of beryllium from the alimentary tract is slight and that beryllium is excreted mainly in the feces. The absorption of beryllium carbonate from the lungs is also very slight. None of the beryllium compounds investigated was dissolved appreciably by serum, while the greatest dilution at which precipitation of protein occurs is one part of beryllium nitrate to 50 of water.

A comparison of the relative toxicities of beryllium, magnesium, and zinc by intraperitoneal injection showed beryllium to be least and zinc most toxic.

Microscopic examination of the tissues of animals which had been exposed to beryllium by inhalation of beryllium dust or fumes, injection of beryllium compounds, or the oral administration of this material, as well as X-ray examination indicates that there is no specific relation between beryllium and rickets and that no consistent pathological change can be attributed to beryllium.

Since no particular toxicity was established for beryllium, it appears that whatever toxicity has been found to occur with the beryllium salts is due to the toxicity of the acid radical such as the fluoride or oxyfluoride, or to an objectionable condition brought about by the hydrolysis of certain of its salts, such as the chloride and sulfate. No safe permissible working standards should be based upon beryllium itself. Safe operating conditions in the preparation of the metal or its alloys must be based upon other considerations than an implied toxicity of beryllium.

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